DEPARTMENT OF PLANT TAXONOMY

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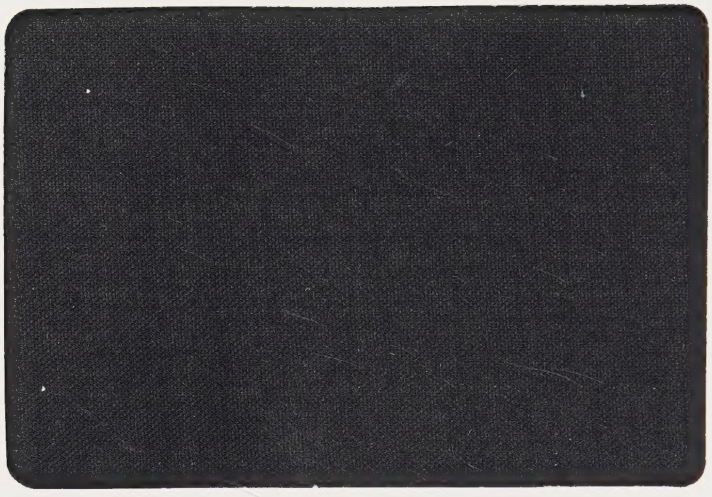

EMBRYOLOGICAL STUDIES IN TYPHACEAE, SPARGANIACEAE AND COMPOSITAE

INGER ASPLUND

1978

AKADEMISK AVHANDLING

för filosofie doktorsexamen i botanik, systematisk inriktning (examinator: Professor Gunnar Harling), som enligt biologisk-geografiska sektionens beslut kommer att offentligt försvaras fredagen den 2 juni 1978 kl. 13.00 i föreläsningssalen, Botaniska Institutionen, Carl Skottsbergs Gata 22, Göteborg.

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EMBRYOLOGICAL STUDIES IN TYPHACEAE, SPARGANIACEAE AND COMPOSITAE

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This survey is based on the following papers which are being referred to by their Roman numerals:

I. Asplund, Inger 1972. Embryological Studies in the Genus Typha. *Svensk Bot. Tidskr. 66*: 1—17.

II. Asplund, Inger 1973. Embryological Studies in the Genus Sparganium. *Svensk Bot. Tidskr. 67*: 177—200.

III. Asplund, Inger 1978. Embryological Studies in Helenieae-Heleninae (Compositae).

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Three families have been assigned to the order Pandanales by most earlier authors: Typhaceae, Sparganiaceae and Pandanaceae. The opinion that the Typhaceae and the Sparganiaceae should be removed from the Pandanales and form an order of their own, the Typhales, has recently been expressed by some authors mentioned in I and II. Takhtajan (1969) also shared this opinion.

The aim of my investigations in the genera *Typha* L. and *Sparganium* L. has been to give information about the embryology and to add some further facts to the discussion of the systematic position of the taxa in question.

The following species of *Typha* and *Sparganium* have been examined: *T. latifolia* L., *T. angustifolia* L. var. *elongata* (Dudley) Wiegand, *S. erectum* L., *S. simplex* Huds., *S. minimum* Wallr., *S. angustifolium* Michx. The material was mainly collected in the Gothenburg area and in the neighbouring provinces of Halland, Bohuslän and Västergötland. Some *Typha* material was collected in the state of Wisconsin, U.S.A., mainly around Milwaukee and Madison.

The results of the studies have revealed a great similarity in all embryological features between *Sparganium* and *Typha*. The wall of the microsporangium is four-layered in both genera. The tapetal cells become multinucleate and the divisions are asynchronous between the cells. The tapetum becomes amoeboid and forms a periplasmodium. The microspore formation follows the successive type. The main part of the pollen tetrads are of isobilateral or decussate type, but linear, T-shaped or tetrahedral types also occur. The microspores remain in tetrads until very late in *Typha latifolia*. The ripe pollen grains are binucleate and have a reticulate exine.

The ovule becomes anatropous, crassinucellate and bitegmic. The ES development is monosporic and follows the Normal type. The four macrospores usually appear in a linear arrangement, but in *Typha latifolia* also T-shaped tetrads are sometimes found. The chalazal macrospore develops into an eight-nucleate ES. The nuclei organize in the usual way forming an

egg apparatus in the micropylar end, three antipodes in the chalazal end and two polar nuclei. The polar nuclei fuse and form a large central nucleus. After fertilization the primary endosperm nucleus moves towards the antipodes where it divides. The wall between the two nuclei divides the ES into two chambers. A Helobialtype of endosperm is developed. The development in the chalazal chamber takes two different courses. In one of these courses no wall formation takes place and thus a coenocytic cell is formed with — in *Sparganium* — up to ten nuclei. In *Typha* as many as 59 nuclei have been found. In the other course wall formation occurs after the nuclear divisions, and a cellular type of endosperm develops with up to 300—400 cells in *Sparganium*. The cells are first uninucleate, later multinucleate. The endosperm nuclei of the micropylar chamber first divide freely but later this part of the endosperm is transformed into multinucleate cells. The divisions in the micropylar and chalazal chambers are asynchronous with respect to one another.

The embryological development in *Typha* and *Sparganium*, regarding the microsporogenesis and male gametogenesis as well as the macrosporogenesis and female gametogenesis, is thus very similar. Important characters such as the development of the tapetum, microspores, antipodes, and endosperm are entirely the same. Especially noticeable is the great similarity in such characters as the development within the chalazal endosperm chamber and the mixture of different microspore tetrad forms, characters that are regarded unusual. The embryology of the Pandanaceae, on the other hand, is very different as pointed out in I p. 15 and II pp. 196—197. The embryological facts together with previously known palynological, anatomical and morphological characters, chromosome numbers, etc. show that the order Pandanales in its usual circumscription is heterogeneous. The families Sparganiaceae and Typhaceae should be removed and put into an order of their own, the Typhales, forming one single family, the Typhaceae, with two subfamilies: the Typhoideae and the Sparganioideae.

Many embryological studies have been made within the Compositae. However, *Gaillardia* is the only genus so far examined within the Helenieae-Heleninae. Since the embryo sac of *Gaillardia* develops according to the Fritillaria type, a type that is very unusual in this family (see III p. 2), an investigation of other genera in the same subtribe would be of interest.

Five genera have been examined: *Chaenactis* DC., *Othake* Raf., *Palafoxia* Lag., *Monolopia* DC. and *Cadiscus* E. Mey. The following species have been studied: *Ch. artemisifolia* A. Gray, *Ch. glabriuscula* DC., *Ch. Fremontii* A. Gray, *O. texanum* (DC.) Bush, *O. sphacelatum* (Nutt. ex Torr.) Rydb., *P. linearis* Lag., *M. gracilens* Gray and *Cadiscus aquaticus* E. Mey. The material mainly originates from plants cultivated in Uppsala Botanical Garden, the Hortus Bergianus in Stockholm and the Botanical Garden in Göteborg.

With minor deviations the microsporogenesis and male gametogenesis follow the normal pattern of development in the family. Four different cell layers are discernable in the sporangium wall after the differentiation of the sporogenous tissue: The epidermis, the endothecium, a middle layer and the tapetum. The microspore formation is of the simultaneous type. The mature pollen grains are three-celled, triporate and more or less sphaeroidal, with a spinuliferous exine. The tapetal cells form a periplasmodium.

The ES development is monosporic in *Chaenactis glabriuscula* and *Ch. Fremontii*. In *Ch. artemisifolia* the ES development is bisporic of the Allium type in about 90% of the material studied and tetrasporic in about 10%. Three antipodes are found in *Ch. artemisifolia*, two in *Ch. glabriuscula*, and two, three or five in *Ch. Fremontii*.

A bisporic ES development is very rare in the Compositae (III p. 18). The type of ES formation is, moreover, usually uniform within a single species and is very often so for all species in the same genus. In *Chaenactis artemisifolia*, however, two ES types have been found even in material from a single specimen.

Rydberg (1914) (III p. 20) divided the genus *Chaenactis* into three sections: Macrocarphus, Acarphaea and Euchaenactis. *Ch. artemisifolia* was placed in the section Acarphaea, and *Ch. glabriuscula* and *Ch. Fremontii* in the section Euchaenactis. The embryological differences found in *Chaenactis* support Rydberg´s proposed subdivision of the genus, as does a chromosome number study by Raven & Kyhos (1961) (III p. 20). However, further studies are needed before a more definite conclusion can be reached.

The genera *Othake* and *Palafoxia* show a tetrasporic ES development of a modified Chrysanthemum parthenium type, except in about 20% of the material of *Othake texanum* in which the ES development is monosporic. A combination of tetrasporic and monosporic ES types as in *O. texanum* is seldom found in normally developed ovules (III p. 19). The number of antipodes found in *O. texanum* is three to six, in *O. sphacelatum* one to six and in *P. linearis* three to four.

Rydberg (1914) and Ammerman Baltzer (1944) refer the genera *Othake* and *Palafoxia* to the subtribe Palafoxiinae ("Palafoxianae"). A third genus, *Polypteris*, is also placed in this subtribe. According to the above-mentioned authors the group thus consists of three distinct, though closely related, genera. Turner & Morris (1976), however, proposed recognition of only a single genus, *Palafoxia*, in agreement with some earlier authors (III p. 21). This proposal is based on morphology, biochemistry and chromosome numbers. The results of the present embryological study lend strong support to their opinion.

Monolopia gracilens has a monosporic ES development with two or three antipodes.

In *Cadiscus aquaticus* ES formation is monosporic and of the Normal type but shows some remarkable differences in detail from the development found in other monosporic Helenieae-Heleninae. The number of antipodes is three or four, and the lower antipode alone occupies 1/3 of the entire space of the ES (III p. 17). During the four-nucleate stage the nuclear constella-

tion is of a special type, as is also later the shape of the antipodal system. The antipodal model is almost certainly a direct result of the special nuclear constellation. Corresponding conditions are not found elsewhere in the Helenieae, but are present in other tribes of the Compositae. Both ES development and the structure of the mature ES of *Cadiscus* fully agree with the conditions described by Afzelius (1924) for several *Senecio* species and by Harling (1950) for some Anthemideae-Anthemidinae genera. The result of my embryological investigations supports the relationship between *Cadiscus* and the Senecioneae-Othonninae proposed by some authors (III p. 22) and based on considerations of morphology, chromosome numbers and phytogeography.

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Embryological Studies in the Genus Typha

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Abstract

Pollen development follows the successive type. The pollen-tetrads comprise about 80 % of the isobilateral type, the rest decussate, tetrahedral, linear or T-shaped. The microspores remain in tetrads until very late in *Typha latifolia*. The ripe pollen grains are 2-nucleate and have a thick reticulate exine. The tapetal cells very soon become multinucleate. Not more than three mitotic divisions of the nuclei are found, but these divisions occur at varying stages of tapetum development and are asynchronous between the cells. The tapetum becomes amoeboid and forms a periplasmodium after the pollentetrads are formed.

Concerning the macrosporogenesis, the archesporial cell arises subepidermally, cuts off one parietal cell and becomes the EMC. The parietal cell divides periclinally and anticlinally. The ovule becomes anatropous and slightly crassinucellate and bitegmic. The EMC divides and forms four megaspores which usually appear in a linear arrangement but in *T. latifolia* also sometimes in a T-shaped arrangement. The chalazal megaspore develops into an embryo sac according to the *Polygonum* type. After fertilization the primary endosperm nucleus moves towards the antipodes where it divides, resulting in a partition of the embryo sac into two chambers. A Helobial type of endosperm is developed and there is no multiplication of the antipodes as has previously been presumed. The development in the chalazal chamber takes two different courses. In one the nucleus divides without subsequent wall formation and thus forms a coenocytic cell with up to ten nuclei. In the other, wall formation occurs after the first nuclear division and a cellular type of endosperm develops. The cells are first uninucleate, later many become multinucleate. The micropylar endosperm first divides freely and later becomes cellular with several multinucleate cells. The divisions in the micropylar and chalazal chambers are asynchronous with each other.

Introduction

According to Engler & Diels (1936) three families are assigned to the order *Pandanales*: *Typhaceae*, *Pandanaceae* and *Sparganiaceae* but during recent decades the relationship between the families has become a matter of dispute. Some authors, e.g. Fagerlind (1940), Harling (1958), Hutchinson (1959), von Guttenberg et al. (1960), U. Müller-Doblies (1969) and D. Müller-Doblies (1969) are of the opinion that the *Typhaceae* and *Sparganiaceae* should be removed from the order *Pandanales*. Among other characters, embryological ones such as the development of the antipodes and the endosperm have played an important role in the discussion of the systematic position of these families. I present here a report with further information with respect to the embryology of the genus *Typha*.

Material and method

The examined material has been collected in Sweden around Gothenburg and in the state of Wisconsin, USA, mainly around Milwaukee and Madison. In Sweden *Typha latifolia* L. was collected and in the USA *Typha latifolia* L. and *T. angustifolia* var. *elongata* (Dudley) Wiegand.

All the material has been prefixed in the field in Carnoy's solution and then put in a fixing solution. Of the different fixatives I have found Karpeschenko to be among the better ones. FAA also gave a fairly good result. After dehydration and infiltration by the TBA method the paraffin method of imbedding was employed according to Johansen (1940). Most of the material was also placed in a vacuum chamber in order to take away as much air as possible from the hairy carpels. The paraffin blocks were cut at a thickness of 8–12 μ and the sections then stained. Among the different staining methods used Heidenhains Iron-Haematoxylin and Fast Green-Safranin-Gentian Violet gave the best result.

Microsporogenesis

The examined species is *Typha latifolia*. The anthers are basally fixed to the filaments and consist of two thecae, each one composed of two microsporangia connected by a well-developed supporting tissue, thus a normal type of stamen for an angiosperm.

When, in the sporangium, the sporogenous tissue is differentiated, it is possible to distinguish epidermis, endothecium, one or two interjacent cell layers of flattened cells and a tapetum consisting usually of one (Fig. 1 A), sometimes of two cell layers as can be seen in Fig. 1 C and D. Occasionally some endothecial cells will divide periclinally. Dietz (1887) mentioned the different cell layers but did not, as Schaffner (1897), refer to any interjacent cell layer between the tapetum and the endothecium. Clausen (1927) mentioned the multinucleate cells of the one- or two-layer thick tapetum while Schaffner observed two nuclei per cell. In the early archespore stage the tapetal cells are uninucleate but very soon the nuclei divide and in the PMC stage I have found the tapetum cells to be 2- to 8-nucleate. Fig. 1 A shows one cell with five nuclei. The nuclei are very distinct and have quite frequently more than one nucleolus. The plasma is homogeneous and dense at this stage. The PMC separate and start to divide. Fig. 1 B shows cells in the metaphase of the heterotypic division, Meiosis I. Most spindles appear directed parallel to the longitudinal axis of the anthers. As normal with respect to a Monocotyledon, there is a wall formation between the dyad cells (Fig. 1 C) and thus the development follows the successive type. The tapetal cells have now increased in size and the plasma begins to vacuolize. The cells are mostly either 2- or 4-nucleate and never more than 8-nucleate. Fig. 1 D shows most of the dyad cells in the anaphase stage of the homotypic division, Meiosis II. The direction of the spindles is now perpendicular to that of the previous division in most of the cases. The result of the meiotic division is pollen-tetrads of mostly the isobilateral type. If the spindles have another direction other types of tetrads may be formed. The microspores remain in tetrads until much later. Now the tapetal cells have increased remarkably in size and are curving towards the centre of the locule (Fig. 1 E). Later the cell walls

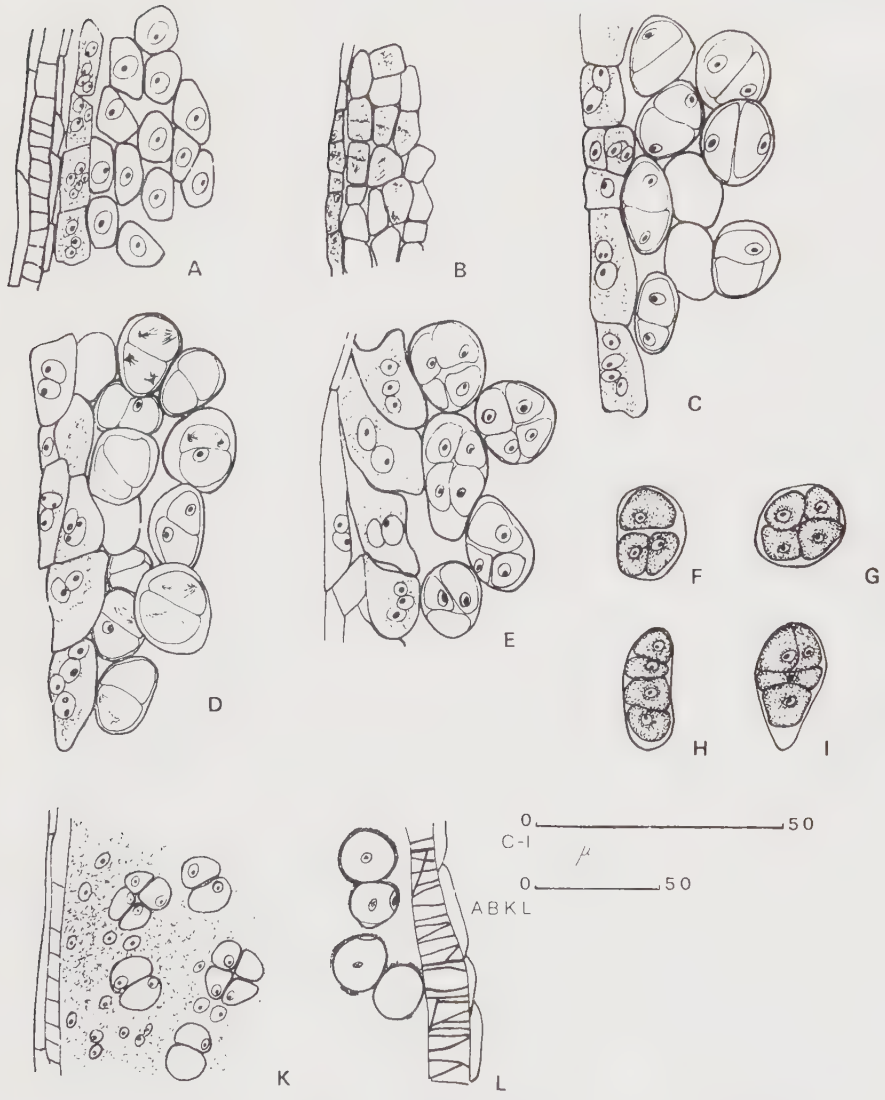


Fig. 1. Microsporogenesis in *Typha latifolia*. The sections of the anthers are longitudinal. *A*, A young anther with pollen mother cells. The tapetal cells are multinucleate. *B*, The pollen mother cells in the metaphase stage (one anaphase) of the heterotypic division, meiosis I. The tapetum multinucleate. *C*, Pollen-dyads. The tapetal cells either 2- or 4-nucleate. *D*, The homotypic division, meiosis II. The tapetal cells are enlarged. *E*, Pollen-tetrads. The enlarged tapetal cells curving in towards the tetrads. *F–I*, Different types of pollen-tetrads. *F*, The decussate type. *G*, The isobilateral type. *H*, The linear type and *I*, The T-shaped type. *K*, A periplasmodium is formed. The pollen still remaining in tetrads. *L*, Mature, 2-nucleate pollen with thick exine. Endothecium with wall thickenings.

disintegrate and the plasma with the nuclei surrounds the pollen-tetrads (Fig. 1 K), forming a periplasmodium. Clausen (1927) classified this type of "amoeboid" tapetum as the *Sparganium* subtype.

The number of nuclei in the tapetal cells seems to vary a great deal. I never found more than eight but this number could be found as early as in the PMC stage just as often as in the tetrad stage. The mitotic divisions in the tapetal cells are not synchronous as can be seen in Fig. 1 C, for instance, where cells with two nuclei and cells with four nuclei appear side by side.

The nuclei of the plasmodium absorb only a little of the stain and consequently they are difficult to detect, only the nucleoli appearing distinctly. The plasma is very coarse and vacuolized. Figs. 1 F, G, H and I show the different positions of the microspores within the tetrads. Approximately 80% are isobilateral (Fig. 1 G), the rest either tetrahedral, decussate (Fig. 1 F), linear (Fig. 1 H) or T-shaped (Fig. 1 I). This very interesting variability of the pollen-tetrads is remarkable and not often mentioned. According to Maheshwari (1950) two or three types of dispositions are described for *Musa*, *Neottia*, *Agave*, *Nicolaia*, *Habenaria*, *Laurus* and *Ottelia*. To this I will add *Sparganium* according to U. Müller-Doblies (1969) and my own not yet published investigation. In *T. latifolia* the microspores adhere in tetrads even at maturity. This has been observed already by Rohrbach (1869), Kronfeld (1887) and Morong (1888). The nature of cohesion in pollen-tetrads of *Typha latifolia* has been examined by Skvarla & Larsson (1963). They have found that "the cohesion is due to the possession of a common exine layer by contiguous grains within the tetrad rather than by pollen mother cell wall retention". They even state that "variations in pollen tetrad form are better explained on the basis of spindle orientations during meiosis II, and the resultant patterns of microspore position rather than on chance disruption of isobilateral microspore tetrads".

The interjacent cells of the anther wall have been pressed together during this process and break down completely as the endothecium enlarges in size. Later the microspores of each tetrad are released and at the same stage the plasmodium disappears. Fig. 1 L shows remnants of the epidermis and the enlarged endothecial cells with a great number of wall thickenings. The ripe pollen grains are 2-nucleate. Their walls have a thin intine and a thick reticulate exine (Fig. 1 L). The plasma is granulated by starch grains.

Macrosporogenesis

Typha latifolia and *T. angustifolia* var. *elongata* are examined and as they follow the same development they are here described together.

The archesporial cell arises subepidermally. It cuts off one parietal cell and becomes the embryo sac mother cell, EMC (Fig. 2 A). The parietal cell divides periclinally and anticlinally as has been shown by Dahlgren (1917).

The ovule becomes anatropous and slightly crassinucellate and possesses two

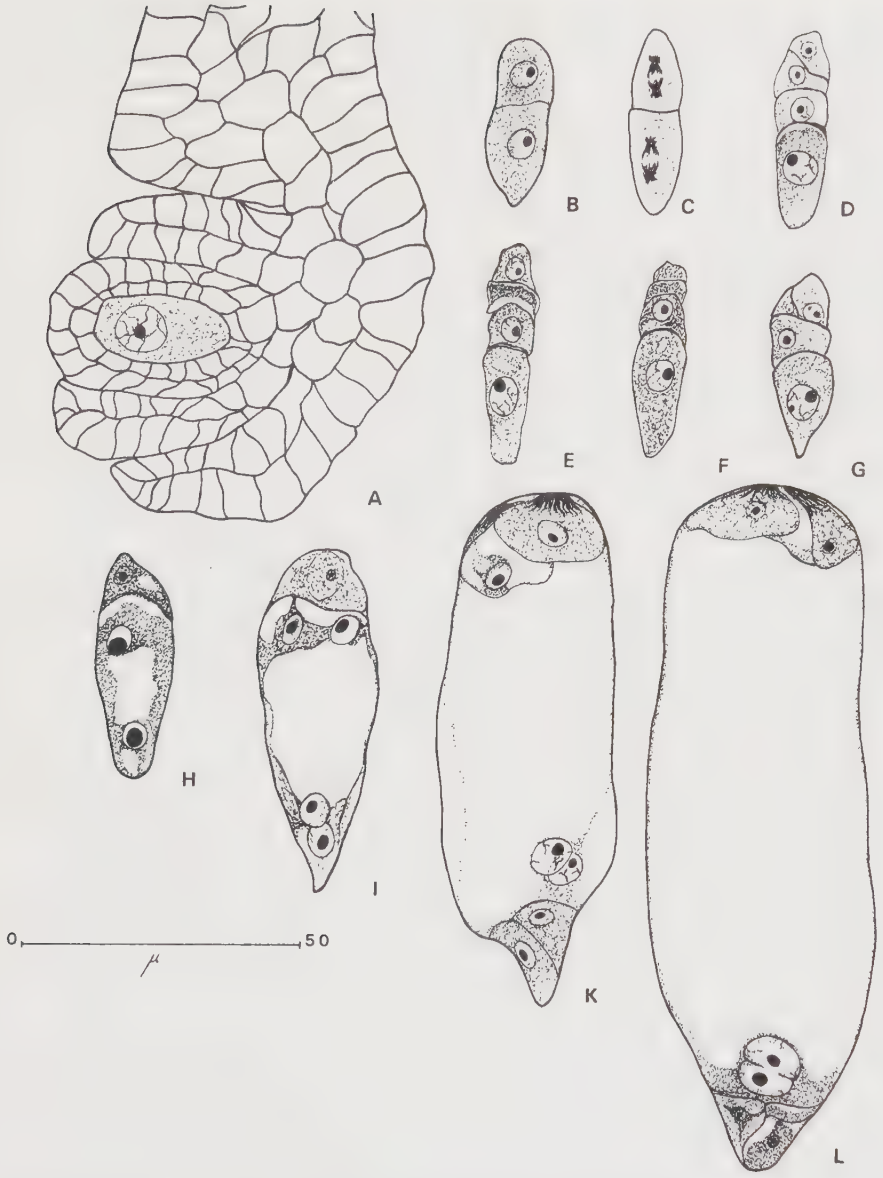


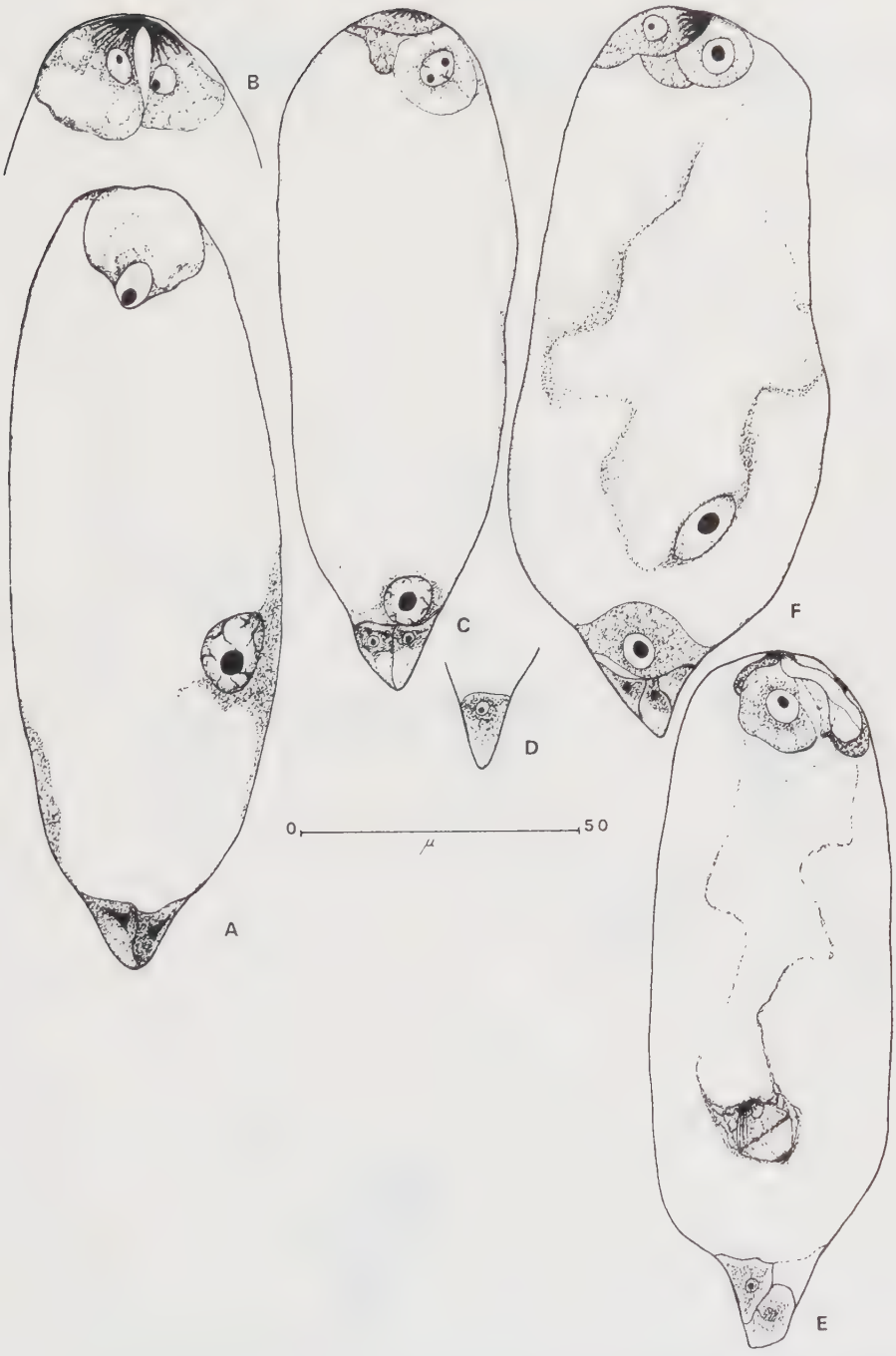
Fig. 2. Megasporogenesis in *Typha latifolia*. *A*, Young anatropous ovule with two lengthening integuments surrounding the nucellus with the EMC. *B*, Dyad cells. *C*, Second division forming tetrad cells. *D*, Tetrad cells in linear arrangement. *E–F*, Later stages. *G*, Tetrad cells in T-shaped arrangement. Megagametogenesis. *H*, Two-nucleate embryo sac with the third megaspore showing. *I*, Four-nucleate embryo sac with the third megaspore still showing. *K*, Eight-nucleate embryo sac of *Polygonum*-type. *L*, The fusion of the two polar nuclei.

integuments each consisting of two cell layers. Together, the two integuments later form the micropyle, contrary to the statements of Dietz (1886 and 1887). Later on, the integuments form what Hegelmaier calls a "Samendeckel", as was found by Dietz op. cit. Probably this functions as an obturator (Fig. 8).

The EMC enlarges mainly by elongation and proceeds through a meiotic division. Two dyad cells are formed (Fig. 2B) and by a second division (Fig. 2C) they give rise to a tetrad. Usually the tetrad cells appear in a linear arrangement (Figs. 2D–F), but in about 10% of the examined ES in *T. latifolia* T-shaped arrangement was found (Fig. 2G). Of the four megaspores the chalazal is the functional one. As Figs. 2E and F show, the micropylar megaspores disintegrate. However, the megaspore next to the chalazal remains for some time. The functional megaspore elongates and enlarges and in the hitherto dense plasma a vacuole appears. The nucleus divides and a 2-nucleate ES is formed with a large vacuole in the centre and one nucleus located in each end of the sac (Fig. 2H). Another division gives rise to a 4-nucleate ES (Fig. 2I). Up to this stage the subchalazal megaspore very often is fully visible but it then disintegrates. The four nuclei of the ES then divide and an 8-nucleate ES is formed according to the *Polygonum*-type. The eight nuclei organize in the usual way (Fig. 2K) to the egg apparatus in the micropylar end, consisting of one egg cell and two synergids, three antipodal cells in the chalazal end and two polar nuclei, one from each pole of the ES. The synergids are pyriform as shown on Fig. 3B, each one with a very distinct nucleus. After the fertilization one appears deformed by the pollen tube (Fig. 3E), but they do not disappear until much later. Fig. 2L shows the fusion of the polar nuclei, located in the lower part of the ES. The fusion nucleus is very large with a large nucleolus (Fig. 3A). After the fertilization the primary endosperm nucleus moves towards the antipodes (Fig. 3C). There it divides (Fig. 3E) and a wall is formed between the two nuclei which results in a partition of the ES in one chalazal and one micropylar chamber (Fig. 3F). Thus a Helobial type of endosperm is developed.

The plasma in the chalazal chamber vacuolizes and soon the nucleus divides (Figs. 4A and B). Later on, the division of the nucleus in the micropylar chamber takes place. Fig. 4C shows two nuclei in each chamber and in Fig. 4E the nuclei in the micropylar chamber are in division. There is no change within the antipodes so far. All three remain in the original condition. The chalazal chamber remains 2-nucleate for quite a while. Figs. 6A and B show two different ES each with eight nuclei in the micropylar chamber but only two nuclei in the chalazal one. In one of the sacs (Fig. 6B), some of the nuclei in the micropylar endosperm have more than one nucleolus. This appears very frequently at this stage of development in the micropylar chamber. The nuclei continue to divide freely, quite asynchronous to the divisions in the basal cham-

Fig. 3. Typha latifolia. A, The fusion nucleus is situated in the central part of the embryo sac. *B*, The synergids from *A*. *C*, The primary endosperm nucleus has moved towards the antipodal cells. *D*, The third antipodal cell not showing in *C*. *E*, The division of the primary endosperm nucleus. *F*, One micropylar and one chalazal chamber are formed.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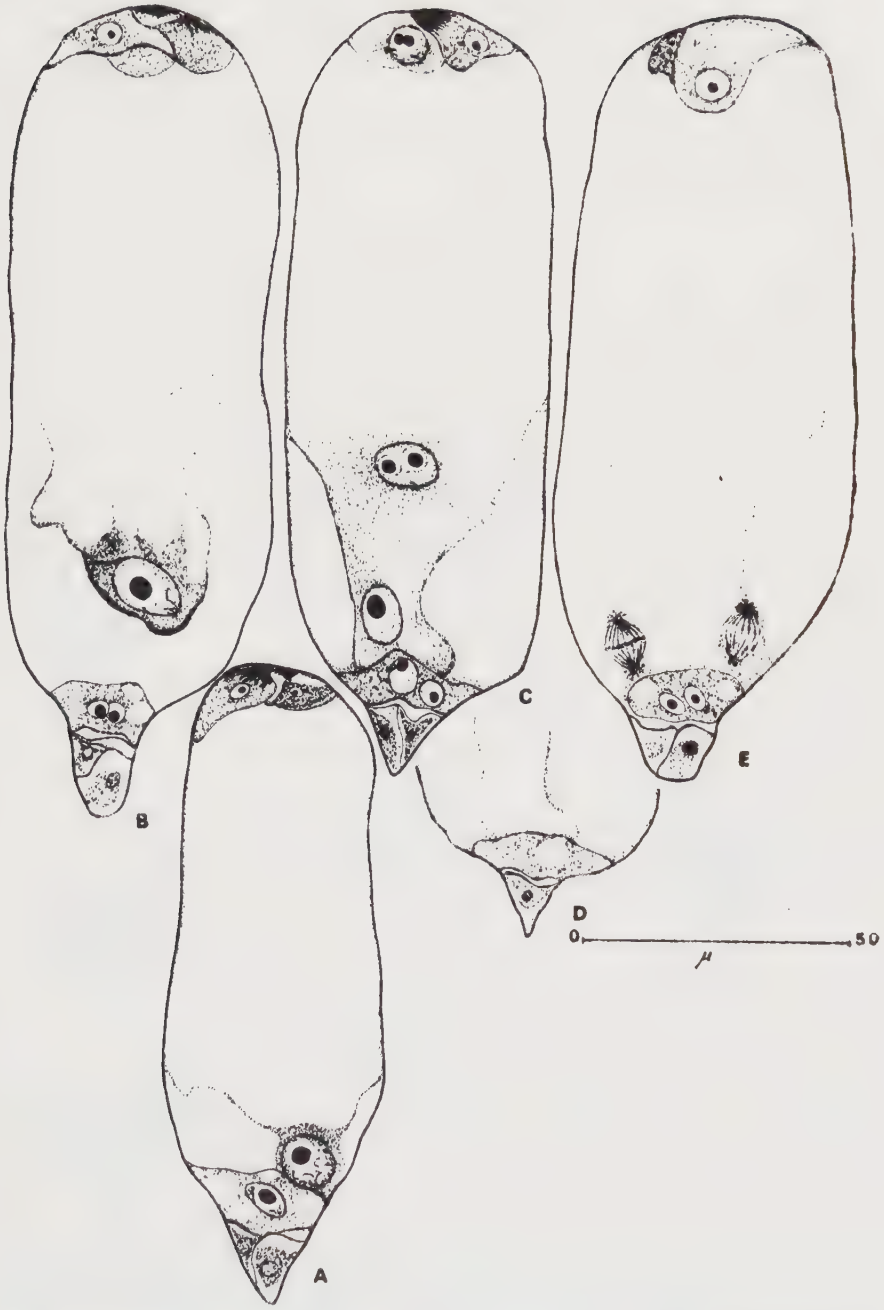

Fig. 4. Typha latifolia. A, Helobial endosperm with one-nucleate chambers. *B*, Two-nucleate chalazal chamber after an asynchronous division. *C*, Micropylar and chalazal chambers two-nucleate. *D*, Third antipodal cell and chalazal chamber from *C*. *E*, The two nuclei in the micropylar chamber in division.

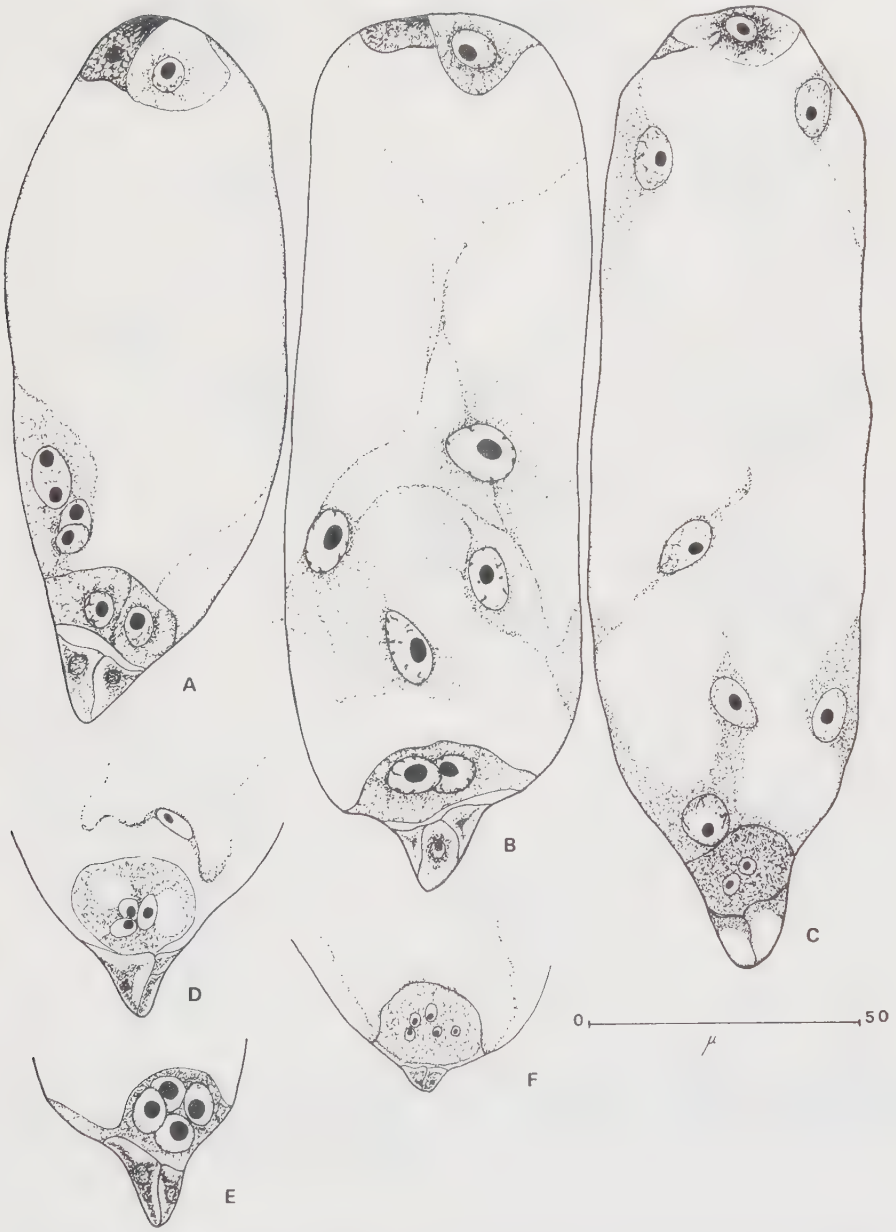

Fig. 5. Typha latifolia. A, Three nuclei in the micropylar chamber, one of which with two nucleoli. Wall formation in the chalazal chamber. *B*, Four nuclei in the micropylar chamber, two in the chalazal. *C*, Six nuclei in the micropylar chamber, two in the chalazal. *D*, Three-nucleate chalazal chamber. *E*, Four-nucleate chalazal chamber. *F*, Five-nucleate chalazal chamber.

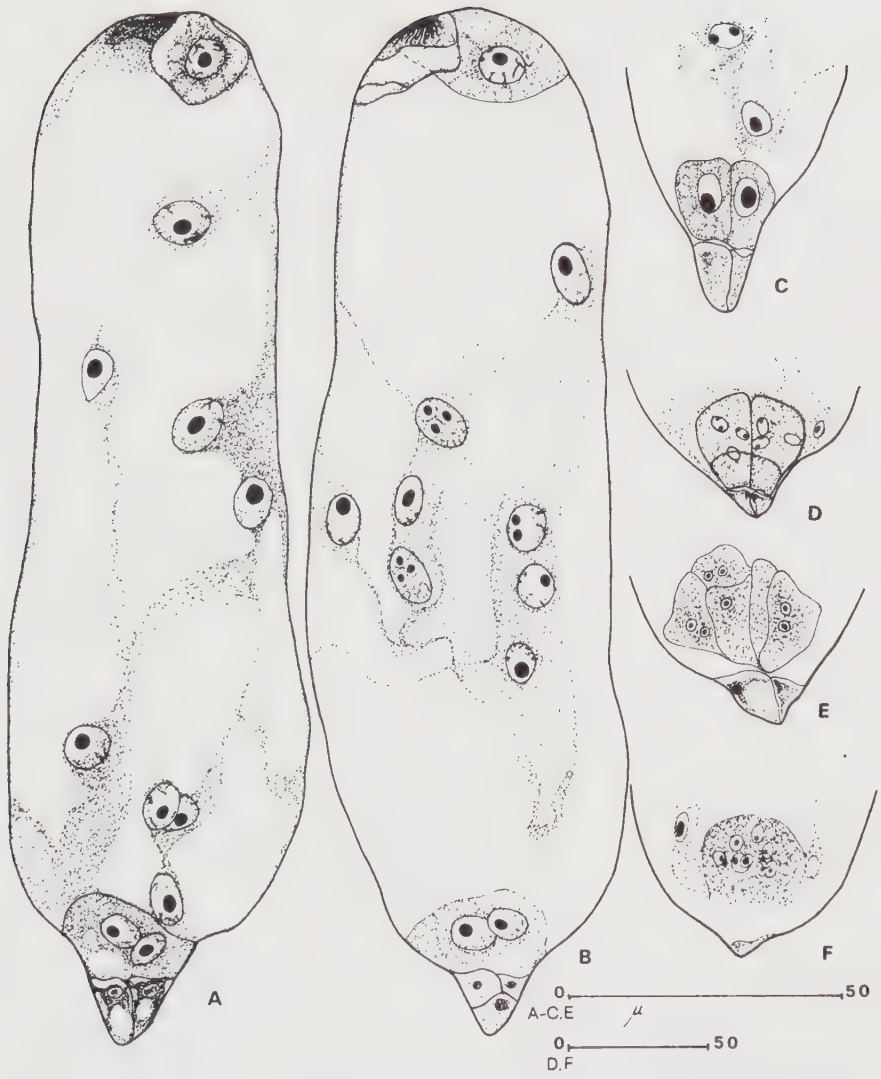

Fig. 6. Typha latifolia. A, Micropylar endosperm with eight nuclei, two nuclei in the chalazal chamber. *B*, As *A*, but some of the micropylar nuclei have more than one nucleolus. *C*, Chalazal chamber with two one-nucleate cells. *D*, Two three-nucleate cells in the chalazal chamber. A third cell showing behind the other two. *E*, Chalazal chamber with five cells, three of which are two-nucleate. *F*, Undivided chalazal chamber with ten nuclei.

ber. Later on, wall formations take place and from being nuclear the micropylar endosperm becomes cellular. The chalazal chamber remains on the 2-nucleate stage until several divisions have taken place in the micropylar chamber. The continuing development is most interesting and follows two different lines. Sometimes a wall for-

Fig. 7. A–B, Typha angustifolia var. *elongata. C, Typha latifolia. A,* The zygote has divided into two cells. Several cells appear in the chalazal chamber. Some of the cells are multinucleate, some just dividing. The antipodes still showing. *B,* Several one-nucleate cells in the chalazal chamber. *C,* Fragments of a coenocytic chalazal chamber. Remnants of the antipodes are noticeable.

Fig. 8. Embryo sac of *Typha latifolia* with the Helobial type of endosperm. The zygote is still undivided.

mation occurs very early at the 2-nucleate stage as can be seen in Fig. 5A. The chalazal chamber has been divided into two cells. They continue to divide as illustrated in Figs. 6C, D and E and soon a large hemispherical mass of cells is formed (Figs. 7A and B). The cells are first uninucleate, but later on they become multinucleate. The second line of development is quite the reverse. No wall formation takes place at all and the nuclei divide freely as shown in Figs. 5D, E and F. The result is a coenocytic chamber with several nuclei in the plasma. The nuclei are normally rather large and distinct at early stages but in older stages with many nuclei they become smaller and less distinct. The coenocytic cell takes a round, hemispherical form.

The antipodal cells maintain their original shape during the whole development just described (Fig. 9) and only when the chalazal endosperm has taken a more hemispherical form do they start to degenerate. However, fragments of the antipodes are quite visible at such a late stage as shown in Fig. 7C. Thus there is no multiplication of the antipodes within the genus *Typha* as has long been considered valid.

Schaffner, the first to present an embryological work with figures of *T. latifolia*, erroneously thought he found an ES development according to the *Lilium* type (now *Adoxa* type) and described the antipodes as only nuclei without any cell walls. Schoenebeck (1924) found three antipodal cells and Graef (1955) presented the development according to the *Polygonum* type. The same was indicated by Dahlgren though he did not see any stages older than the tetrad. Schoenebeck mentioned that the

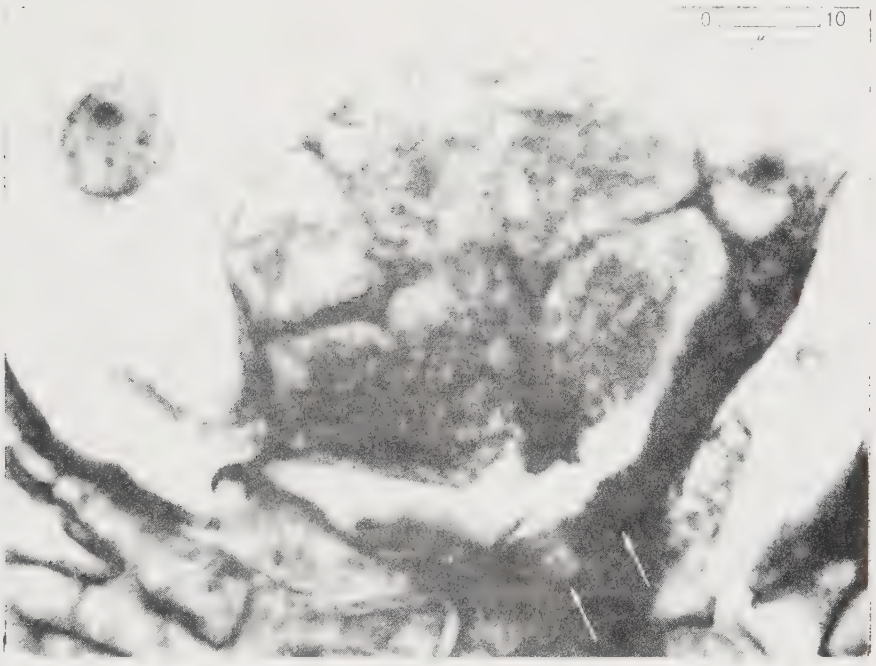

Fig. 9. Typha latifolia. The chalazal chamber with several cells. The arrows point to two of the antipodes.

primary endosperm nucleus divides after the fertilization and a protoplasmatic wall covering appears. He discovered no basal cell and thus stated wrongly that there was a nuclear type of endosperm.

Swamy and Parameswaran (1962) gave a summary statement of the variation of the Helobial type of endosperm. For monocotyledons they stated among other things:

that the endosperm in the micropylar chamber is first nuclear and later becomes cellular;

that if the nucleus in the chalazal chamber divides the division occurs freely;

that if a coenocytic chalazal chamber is formed the first few divisions in the micropylar and chalazal chambers are synchronous; and

that if in the chalazal chamber a cell wall is formed it appears rather late and is delayed also in comparison to the time of wall formation in the micropylar chamber.

For *Typha* the first statement is correct. According to the second this is always true for the first division but for the following ones only according to one of the two above-mentioned lines of development in the chalazal chamber. There is no synchronous division as mentioned in the third statement and finally the first wall formation is delayed but not in comparison with the conditions in the micropylar chamber.

The variability in the Helobial type of endosperm is also discussed by Maheshwari

(1950). He gives some examples where the division in the chalazal chamber is accompanied by wall formations. One is the report by Juel (1907) according to *Saxifraga granulata*. The first few divisions may be free nuclear with wall formations occurring later on. This type of development is regarded as quite unusual. Among the cases first reported was *Heloniopsis breviscapa* (Ono 1927).

In *Typha* the first division in the chalazal chamber always seems to be a free nuclear division but then very frequently a wall formation appears. In later stages I have found nearly as many ES with a coenocytic cell as with a cell formation in the basal chamber. Not until I found an embryo sac with a fully developed embryo and with the remains of a coenocytic chalazal chamber did I know that my suspicions of development according to two different ways were accurate. I have found the same development in the Swedish as in the American material. I have mainly concentrated my investigations on *T. latifolia* and there I will draw the conclusion that the two different courses of development in the chalazal chamber exist in about the same degree. I have never seen more than ten nuclei in the coenocytic cell. At the beginning they are very large but after several divisions the daughter nuclei are small and not very distinct. They disintegrate after a while, leaving a large plasmatic cell. Usually there are around twenty cells in the cellular chalazal chamber. At a late stage many of them have more than one nucleus. The micropylar endosperm is first nuclear and later becomes cellular with several multinucleate cells.

Discussion

Since Campbell (1899) published a paper on an embryological investigation within the genus *Sparganium* and there stated that a multiplication of the antipodes existed, there has been some speculation as to whether the same thing occurred within the genus *Typha* or not. Schürhoff took up the question in his attempt to make a comparison between the orders *Pandanales* and *Helobiae*:

Pandanales	*Helobiae*
kein Mikrosporenperiplasmodium	Periplasmodium
zweikernige Pollen	dreikernige Pollen
kein Basalapparat	Basalapparat
Vermehrung der Antipoden (*Typhaceae*?)	keine Antipodenvermehrung
keine grosse Suspensorzelle	riesenhafte Suspensorzelle

Schoenebeck studied the ES development in *Typha* and confirmed the expectation according to multiplication of the antipodes. His figures are most doubtful and show a very conspicuous form of the chalazal end of the sacs. The cells he points out as antipodes originating from a multiplication of the original three antipodes are probably some neighbouring somatic cells together with the antipodes and some cells from the cellular chalazal chamber.

In a preliminary report (1968) I indicated that *Typha latifolia* had a Helobial type

of endosperm and after further investigations within the genus I have found this very interesting development within the chalazal chamber. As I already stated (Asplund 1958), the genus *Sparganium* also has a Helobial type of endosperm. Ute Müller-Doblies (1969) found the same thing and no doubt there is a great similarity in the ES development as well as in the microsporogenesis between the genera *Typha* and *Sparganium*, which I will take up in a coming paper. According to the result of this investigation there is not much left of the separating characteristics which Schürhoff mentioned for *Pandanales* and *Helobiae*.

In *Pandanaceae*, the third family assigned to *Pandanales*, are usually included *Pandanus*, *Freycinetia* and *Sararanga*. The embryological conditions are only known for the two first-mentioned genera. The ES of *Pandanus* was described by Campbell (1909, 1910 and 1911) but first fully investigated by Fagerlind (1940). In some species of the genus, Fagerlind observed an increase in number of the antipodal cells through a migration of nuclei from the somatic neighbouring cells. The plasma and vacuoles of these cells also penetrated into the chalazal or lateral part of the sac. This is a most interesting phenomenon. Some stages of the ES development in *Freycinetia* were also reported by Fagerlind. Some years later his pupil Strömberg (1956) made a more complete examination of this genus and reported no antipodal multiplication or migration of nuclei. Instead the chalazal neighbouring cells degenerate entirely and remain only as fragments. Even the antipodes degenerate early. Only a few embryological facts supported a connection with *Pandanus*, such as the construction of the integuments, the development of the nucellar epidermis and the hypostasis. Both genera have an anatropous, bitegmic and crassinucellar ovule. In *Pandanus* the micropyle is formed by the inner integument only, whereas in *Freycinetia* both integuments are involved. The development in *Freycinetia* and *Pandanus* is described as following the *Polygonum* type. They both have a nuclear endosperm formation.

The embryological development in the genus *Typha* described above shows that only some few characters are the same for the *Typhaceae* and the *Pandanaceae*. Important characters such as the development of the antipodes and the endosperm are entirely the reverse. The pollen development is not yet fully investigated for the *Pandanaceae* and thus a comparison is not possible. The chromosome number for *Typha* is reported as $n = 15$ by, among others, Roscoe (1927), Clausen (1927) and Harada (1947). The same number is found for *Sparganium* (Löve, Á. & Löve, D. 1948) but for *Pandanus* $n = 30$ and $n = 32$ are reported (Harada 1947 and Tjio 1948). Thus the above-described embryological conditions conclusively underline the disrelation suspected by some authors within *Pandanales* between the *Pandanaceae* on the one hand and the *Typhaceae* and the *Sparganiaceae* on the other. The last-named families should therefore definitively be removed from the *Pandanales* and put into an order of its own, the *Typhales*.

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Abstract

Five species of *Sparganium* have been studied. The wall of the microsporangium is four-layered. The tapetal cells become multinucleate and the divisions are asynchronous between the cells. The tapetum forms a periplasmodium. The microspore formation follows the successive type. The main part of the pollen-tetrads are of either isobilateral or decussate type, the rest of linear, T-shaped, or tetrahedral type. The ripe pollen grains are 2-nucleate and have a reticulate exine. The ovule becomes anatropous, crassinucellate, and bitegmic. The development of the embryo sac is monosporic and follows the *Polygonum* type. The four megaspores appear in a linear arrangement and the chalazal one develops into an embryo sac. The secondary nucleus is often situated in the neighbourhood of the egg apparatus and after fertilization the primary endosperm nucleus moves towards the antipodes, where it divides, forming a partition of the embryo sac into two chambers. A Helobial type of endosperm is developed and, as in *Typha*, the development in the chalazal chamber takes two different courses. In one of these courses wall formation occurs after the second nuclear division and a cellular type of endosperm develops with up to 300–400 cells. The cells are first uninucleate, later multinucleate. In the other no wall formation takes place and a coenocytic cell is formed. As many as 59 nuclei have been counted. The endosperm in the micropylar chamber first develops according to the nuclear type, later it becomes cellular with multinucleate cells. The divisions in the micropylar and chalazal chambers are asynchronous in relation to each other.

Introduction

There is a lot of dispute about the relationships between the families *Typhaceae*, *Pandanaceae* and *Sparganiaceae*, all assigned by Eckardt (1964) and most other authors to the order *Pandanales*. As mentioned in a previous paper on the embryological development in the genus *Typha* (Asplund 1972), there are some authors, e.g. Fagerlind (1940), Harling (1958), Hutchinson (1959), von Guttenberg et al. (1960), U. Müller-Doblies (1969), and D. Müller-Doblies (1969) who are of the opinion that the *Typhaceae* and the *Sparganiaceae* should be removed from the order *Pandanales*. Lee & Fairbrothers express similar thoughts in a newly (1972) presented paper. There are many different characters to consider in the discussion of the systematic position of the taxa in question. This report hopes to add some facts to this discussion as it will give further information according to the embryology

of the genus *Sparganium*. Some results were already presented in a preliminary report 1968. Every step in the development will also be compared with the corresponding one within the genus *Typha*.

Material and Method

The following species of the genus *Sparganium* have been examined: *S. erectum* L., *S. simplex* Huds., *S. minimum* Wallr., *S. glomeratum* Laest., and *S. angustifolium* Michx. The material has been collected in the Gothenburg area and in the neighbouring provinces of Bohuslän, Halland, and Västergötland.

The method is the same as has been used for the genus *Typha*, and will here be given a short description. All the material has been prefixed in Carnoy's solution before being put in a final fixing solution, this for the purpose of softening the very hard pericarp. I have mainly used Karpeschenko's fixative, and the main part of the fixed material was then placed in a vacuum chamber in order to take away as much air as possible. After dehydration and infiltration by the TBA method the paraffin method of imbedding was employed according to Johansen (1940). The paraffin blocks were cut on a rotary microtome at a thickness of 12–22 μ and then stained in Fast green–Safranin–Gentian Violet or Heidenhain's Iron-Haematoxylin–Orange G.

Microsporogenesis and Male Gametogenesis

The examined species are *Sparganium erectum* L. and *S. simplex* Huds. *Sparganium* has a normal type of stamen for an angiosperm and, like *Typha*, a well-developed supporting tissue connecting the two microsporangia of each theca. After the differentiation of the sporogenous tissue one can discern four different cell layers of the sporangium wall: the epidermis, the endothecium, a middle layer, and the tapetum. Similar to the conditions within the genus *Typha* it happens occasionally that some cells of the endothecium divide periclinally, and quite frequently that the middle layer and the tapetum are two cell layers thick in some local places as the figures show. (Cf. *Typha* in Asplund 1972, Figs. 1 C and 1 D.) These observations correspond to those of U. Müller-Doblies (1969). In a paper of 1927 Clausen described the tapetum of *S. simplex* as being "mehrschichtiges". No figure corroborates his statement, however, but I doubt that he meant the whole tapetum.

The tapetal cells are mostly uninucleate during the early archespore stage (Fig. 1 A), but some cells may be 2-nucleate (Fig. 1 B). The plasma of the cells is dense and appears to be quite homogeneous. The tapetal cells now increase in size, the plasma vacuolizes, and at the time of the PMC stage the main part of the cells are 2-nucleate (Fig. 1 C). The nuclei of the cells do not divide synchronously and thus there are in this stage some cells that are still uninucleate and some that are even 4-nucleate.

At the end of the prophase of the Meiosis I another division of the tapetal nuclei

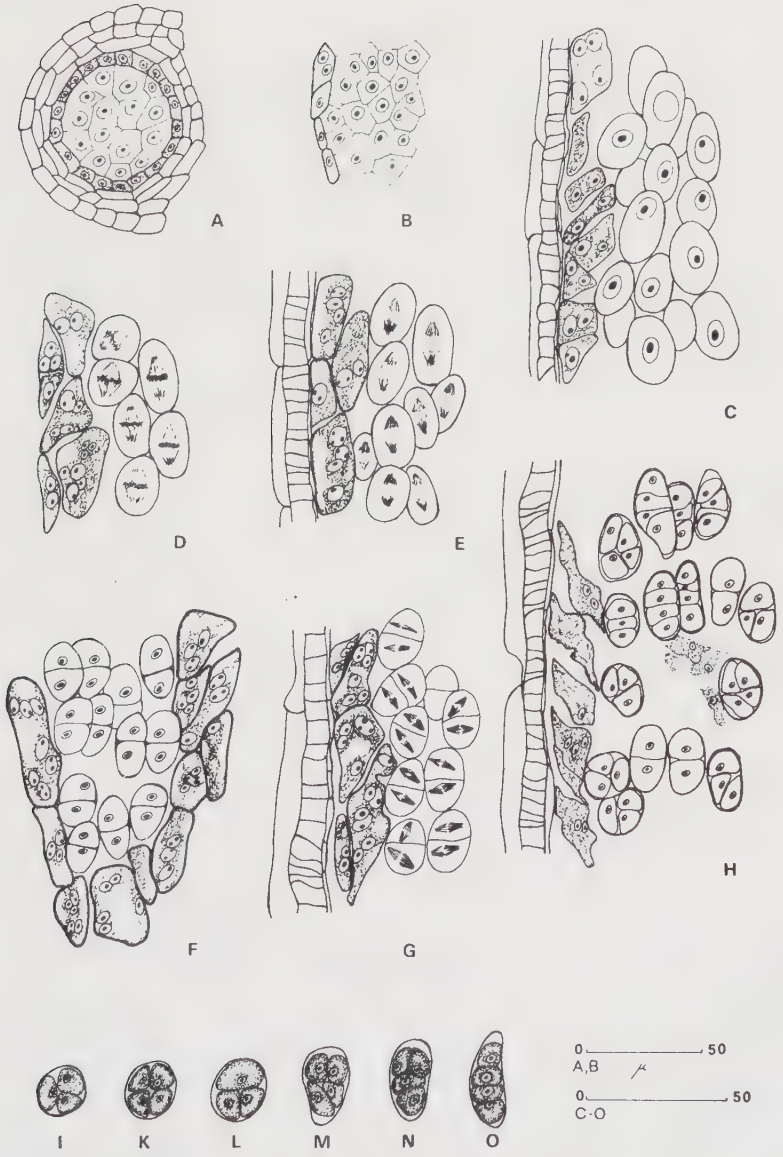

Fig. 1. Microsporogenesis in *Sparganium erectum*. The sections of the anthers are in A and B transversal, in C–H longitudinal. (A) Early archespore stage with one-nucleate tapetal cells. (B) One cell with two nuclei on the same early stage. (C) A young anther with pollen mother cells. The tapetal cells one- to four-nucleate. (D) The pollen mother cells in metaphase stage of the heterotype division, Meiosis I. One tapetal cell with seven nuclei. (E) The anaphase stage of the same division. (F) Pollen-dyads. The tapetum multinucleate. (G) The anaphase stage of the homotypic division, Meiosis II. The tapetal cells enlarged. (H) Pollen-tetrads. The tapetal cells curving in towards the centre of the loculus. (I–O). Different types of pollen-tetrads: (I) The tetrahedral type. (K) The isobilateral type. (L) The decussate type. (M) An oblique isobilateral type. (N) The T-shaped type. (O) The linear type.

takes place and the cells become 4-nucleate. A third division will follow and in the metaphase stage of the heterotypic division some cells are 8-nucleate. Fig. 1 D shows one cell with 7 nuclei and the rest either 4- or 2-nucleate. This illustrates the asynchronous divisions between the cells. The spindles of the PMC are mostly oriented longitudinally to the axis of the anthers. Fig. 1 E shows the anaphase stage with large, vacuolized tapetal cells. Worth noting is that the nuclei of the different cells and even within one and the same cell often are of different sizes. They contain also frequently more than one nucleolus. The development follows the successive type as there is a wall formation between the dyad nuclei as can be seen on Fig. 1 F. This is the normal type of development for a monocotyledon. In the homotypic division, the Meiosis II, the spindles are mostly oriented perpendicularly to those of the heterotypic division, the Meiosis I.

There are, however, many exceptions and among those there is a big variation of the size of the angle between the spindles of the dyad nuclei. This is illustrated on Fig. 1 G which shows the dyad nuclei in the anaphase stage. The type of pollen tetrads formed is dependent upon the direction of the spindles. The very enlarged and mostly 8-nucleate tapetal cells are now starting to curve towards the centre of the loculus. The nuclei are still very distinct. The plasma is quite vacuolized and coarse. When the tetrads are formed, as seen on Fig. 1 H, the tapetal nuclei begin to degenerate, the cell walls start to disintegrate, and the plasma and the nuclei will float in and surround the almost separated tetrad cells, the microspores, forming a periplasmodium. The nuclei take up very little dye and are hard to distinguish. Later as seen on Fig. 2 A the separated microspores become 2-nucleate. They are still surrounded by the periplasmodium. This has disappeared when the pollen grains are ripe and the reticulate exine is fully developed (Fig. 2 B). In *Typha* the periplasmodium is formed already while the microspores remain in tetrads.

Tischler (1915) found in *S. erectum* the tapetal cells to be 2-nucleate at the time of the synapsis and 4-nucleate at the tetrad stage. Clausen (op. cit.), however, described 4-nucleate cells as early as in the premeiotic stage for *S. simplex*. I have not found any differences in the development between the two species mentioned above and my observations correspond to those made by U. Müller-Doblies (op. cit.). Like her, I think that Tischler and Clausen were not aware of the asynchronous divisions between the tapetal cells and took the highest number of nuclei for the normal condition of the stage in question.

For *Typha latifolia* I have found the tapetal cells to be 2- to 8-nucleate. The mitotic divisions occur in varying stages of the tapetum development and are as in *Sparganium* asynchronous between the cells. They are, however, occurring at more irregular intervals in *Typha*. I have never, as in *Typha*, found a third division to have taken place as early as at the PMC stage in *Sparganium*.

The very high number of nuclei in the tapetal cells found in both *Typha* and *Sparganium* seems to be very unusual in the monocotyledons. In a collation made by

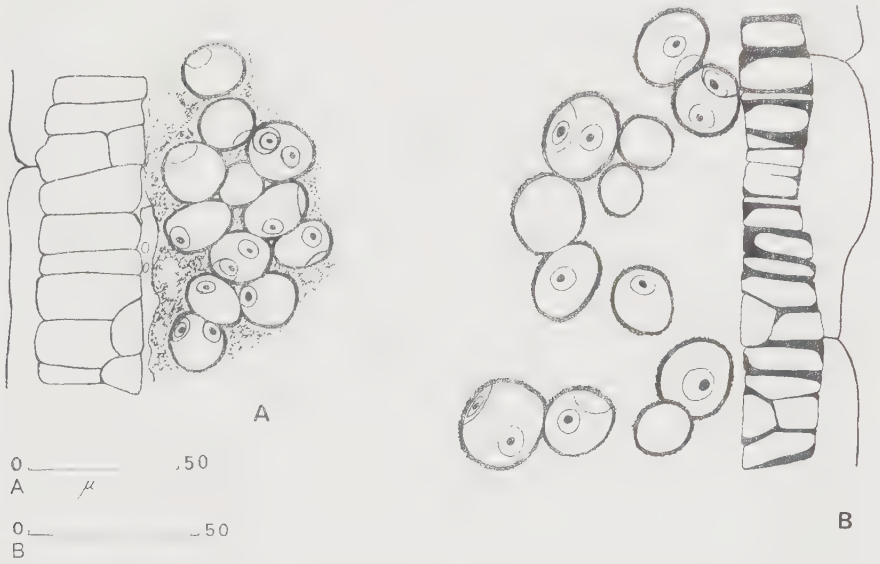

Fig. 2. Microgametogenesis. The sections of the anthers are longitudinal. (A) *Sparganium erectum*. (B) *S. simplex*. (A) Two-nucleate microspores. A periplasmodium is formed. (B) Mature pollen with thick exine. Endothecium with wall thickenings.

Wunderlich (1954) no such development is reported. Also with respect to the formation of the periplasmodium there is a disagreement in opinion between Tischler and Clausen. The former stated (op. cit. p. 74) that when the plasma surrounded the young microspores he could still see "die trennenden Plasmawände, die vorher die einzelnen Zellen begrenzten. Die Zellulosemembranen sind gelöst, aber die Verschmelzung der Zellinhalte zu einem typischen Periplasmodium ist nicht mehr eingetreten." According to the latter the tapetal cells start to curve towards the centre of the sporangium as early as during the Meiosis I and during the tetrad stage the plasma surrounds the pollen tetrads. U. Müller-Doblies's description corresponds to my own observations and is thus not in keeping with either Tischler's or Clausen's.

According to the development described above the walls of the mostly 8-nucleate tapetal cells start to disintegrate during the postmeiotic phase and according to Schnarf (1923, 1929) and Tischler (1915) the periplasmodium formed is false as the nuclei are more or less degenerated. It surrounds the tetrads at the time of separating of the microspores. Carniel (1952, 1963) distinguishes the "amoeboid" tapetum from the periplasmodial type, and cites some gliding transition forms after Chiarugi (1927). Like U. Müller-Doblies I agree with Steffen & Landmann (1957) not to separate the "amoeboid" type from the one forming a false periplasmodium as a type of its own. There are far too many transitional forms to make a too fine differentiation sensible. Within *Typha* the nuclei of the periplasmodium degenerate a little later and the peri-

plasmodium surrounds the tetrads. As the microspores remain in tetrads until very late, the periplasmodium has almost disappeared when the pollen grains have separated.

The homotypic division results in the formation of pollen tetrads. Because of the great variability of the direction of the spindles the tetrads are of the most different types (Fig. 1 H, cp. also Skvarla & Larson 1963). The main part are of either the isobilateral (Fig. 1 K) or the decussate type (Fig. 1 L); the rest of linear (Fig. 1 O), T-shaped (Fig. 1 N) or tetrahedral type (Fig. 1 I). Quite often I have found tetrads of different types to be more or less oblique (Fig. 1 M shows an oblique isobilateral tetrad). Sometimes this is very striking as shown in Fig. 1 H that also illustrates the most considerable variation of tetrad forms. Among the tetrads in Fig. 1 H also three dyads can be seen. Thus the divisions are asynchronous even concerning the PMC.

The development described is very much like the one observed in the genus *Typha*. The latter genus has the same variability of pollen-tetrad types, but not in that high degree as observed in *Sparganium*. In *Typha* most of the tetrads, about 80%, belong to the isobilateral type. The remarkable similarity in this interesting character is really worth noting as it is considered quite unusual. According to Maheshwari (1950) two or three tetrad types are reported for *Musa*, *Neottia*, *Agave*, *Nicolaia*, *Habenaria*, *Laurus* and *Ottelia*.

When the microspores are released from the tetrads and surrounded by the periplasmodium their nuclei divide mitotically, which leads to the formation of 2-nucleate pollen grains. Fig 2 B shows the ripe pollen grains with a thick reticulate exine. The periplasmodium has now disappeared and so have the cells of the middle layer. They have been pressed together by the enlarging tapetal and endothecial cells and completely broken down. At this late stage the large endothecial cells have got a great number of wall thickenings as can be seen on the figure. The same figure also shows the remnants of the epidermis. Two-nucleate ripe pollen grains have previously been reported for *Sparganium* by Elfving (1879), Brewbaker (1967), and U. Müller-Doblies (1969). Two-nucleate ripe pollen grains have been reported for *Typha* by Brewbaker (1967) and myself (1972). The plasma of the pollen grains is granulated by starch grains, a character similar to what I have found in *Typha*.

Pollen Morphology

Erdtman (1952) described the pollen grains of the *Sparganiaceae* to be "ulcerate, spheroidal. Sexine as thick as nexine or usually slightly thicker, reticulate. Ulcus faintly delimited". He also reports: "Pollen grains similar to (or undistinguishable from) those in *Sparganiaceae* occur in *Typhaceae*."

To find out the structure of the surface of the pollen grain wall within *Pandanales* some scanning pictures were taken of pollen grains from the genera *Sparganium*, *Typha*, *Pandanus*, and *Freycinetia*. Plate I a and b show pollen grains from *Sparganium*

erectum and *S. simplex* respectively. On Plate I a can be seen the spheroidal form of the pollen with the ulcus. Plate I b illustrates the fine reticulate pattern of the wall surface. It was of great interest to find the high correspondence between *Sparganium* and *Typha*, both regarding the shape of the pollen grains and the pattern of the wall surface. The latter genus can be seen on Plate II where II a shows the spheroidal form of the pollen grains of *Typha latifolia*, and II b a detail of the wall structure around the ulcus of the same species.

The grains of the genera *Pandanus* and *Freycinetia* are different from those of *Sparganium* and *Typha*. Plate III a illustrates a pollen grain of *Pandanus fascicularis* Lamk. The shape is more or less spheroidal and the surface spinuliferous. The last mentioned character is best illustrated on Plate III b, which shows a part of the wall surface at a higher magnification. On Plate IVa a pollen grain of *Freycinetia Banksii* A. Cunn. can be seen. The shape is here more ellipsoidal and the wall surface psilate. Plate IV b shows the ulceroid—faintly sulcoid aperture and the psilate wall surface.

Macrosporogenesis and Female Gametogenesis

The examined species are *Sparganium erectum* L., *S. simplex* Huds., *S. minimum* Wallr., *S. glomeratum* Laest., and *S. angustifolium* Michx. They follow the same development and are here described together.

The ovule becomes anatropous, crassinucellate, and bitegmic. Both integuments consist of two cell layers and together they later form the micropyle, contrary to the statements of Dietz (1886 and 1887) but in agreement with those of U. Müller-Doblies (op. cit.). The integuments later on form what Hegelmaier calls a "Samendeckel", as was found by Dietz (op. cit.) (Fig. 10B). As in *Typha* the archesporial cell arises subepidermally. It cuts off one parietal cell and becomes the embryo sac mother cell, the EMC. The parietal cell divides periclinally and anticlinally and after at least one, sometimes two divisions of the epidermis, the EMC is covered by usually 4–5, occasionally up to 8 cell layers. I have not found any fixed number in any in the examined species. Fig. 3A shows an EMC covered by four cell layers, of which the outer two form a slightly developed nucellar cap.

The EMC enlarges mainly by elongation and divides meiotically. The result of the first division is two dyad cells (Fig. 3B), which contrary to *Typha* are unequal in size. The chalazal one is larger than the micropylar one. After another division a linear tetrad is formed (Fig. 3C). This is the only arrangement of the macrospores that I have found. In *Typha latifolia* about 10% have a T-shaped arrangement. The basal macrospore, the functional one, enlarges through elongation and will form the embryo sac, the ES. The other three macrospores soon degenerate. However, the macrospore next to the basal one will remain for some time. The nucleus of the functional macrospore divides (Fig. 3D) forming a 2-nucleate ES and, as Fig. 3E shows,

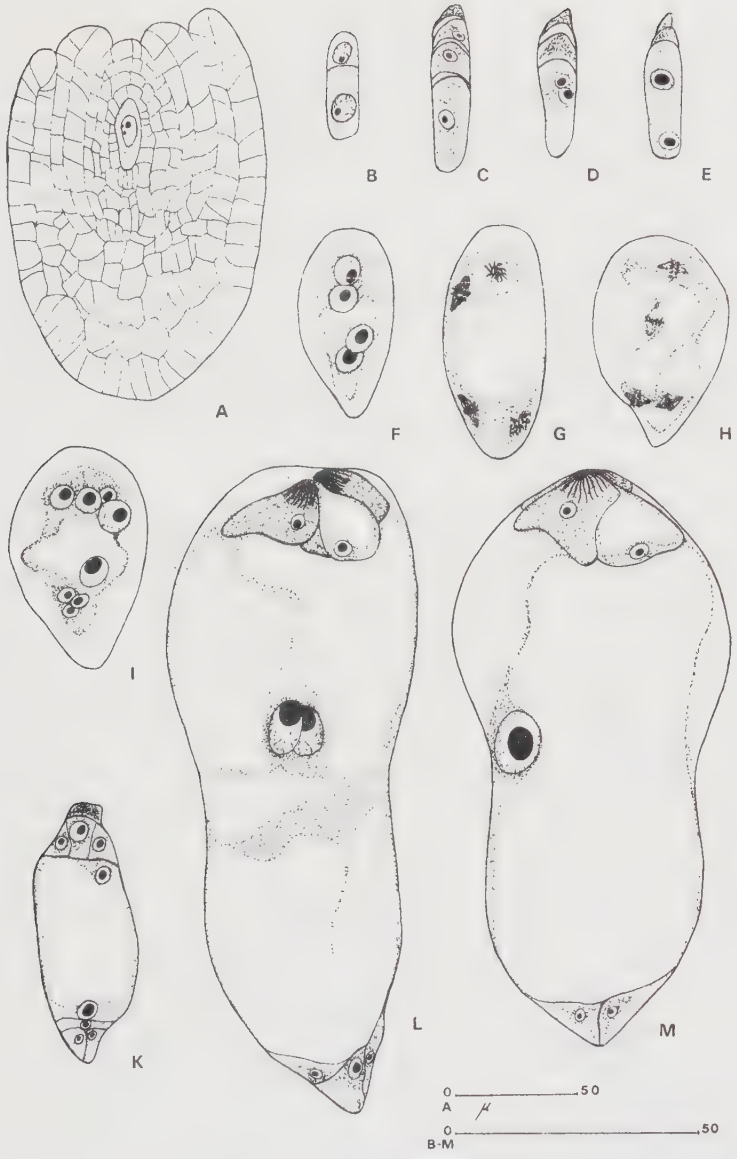

Fig. 3. (A–E, K–M) *Sparganium erectum.* (F, H, I) *S. simplex.* (G) *S. glomeratum.* Macrosporogenesis: (A) Young anatropous, crassinucellate and bitegmic ovule with the EMC. (B) Dyad cells. (C) Tetrad cells in linear arrangement. Macrogametogenesis: (D) The nucleus of the chalazal macrospore has just divided. (E) Two-nucleate ES with the second and third macrospores showing. (F) Four-nucleate ES. (G, H) The four nuclei in division. Two of the spindles in G oriented in another plane than the others. (I) Eight-nucleate ES. Notice the large nuclei that will become the polar nuclei. (K) Newly organized eight-nucleate ES with the third macrospore still showing. (L) Eight-nucleate ES of *Polygonum* type with the two polar nuclei side by side. (M) The fusion nucleus situated in the central part of the ES.

the nuclei will soon be located one in each end of the sac with a vacuole in between. Another division gives rise to a 4-nucleate ES (Fig. 3F). The four nuclei divide (Figs. 3G and H) and an 8-nucleate ES is formed according to the *Polygonum* type. Fig. 3I shows the eight nuclei shortly after the division. Notice the large polar nuclei. A newly formed ES with cell walls around the upper three and the lower three nuclei and in between them the two polar nuclei can be seen on Fig. 3K. On this figure the subchalazal macrospore is still visible. Usually it disintegrates after the 4-nucleate stage. The ES organize in the usual way, Fig. 3L, forming an egg apparatus in the micropylar end, consisting of two synergids and one egg cell, and three antipodes in the chalazal end. The synergids are pyriform, each one with a distinct nucleus. During the fertilization one of the synergids may become slightly deformed by the pollen tube, but none of them disappears until much later. The polar nuclei are located side by side in the central part of the sac. They fuse forming a large secondary nucleus with a large nucleolus (Fig. 3M). In the examined material I have once found two competing ES in *S. erectum*, each one fully developed and of about normal size (Fig. 4A). Their origin might have been the subchalazal and the chalazal macrospores or two archesporial cells. The secondary nucleus is often situated in the neighbourhood of the egg apparatus (Fig. 4B), but after the fertilization the primary endosperm nucleus moves towards the antipodes (Figs. 4C and D) where it divides (Fig. 5A). The division gives rise to a partition of the ES in one chalazal and one micropylar chamber as a wall is formed between the two nuclei. Thus the development is in accordance with the Helobial type of endosperm. The location of the secondary nucleus near the egg apparatus was only occasionally found in *S. erectum*, but very frequently in all the other species examined. This statement is contradictory to that of U. Müller-Doblies, who locates both the polar nuclei and the secondary nucleus in the central part of the ES. She gives only one exception from this of the 15 ES of the stages in question she refers to. This statement of hers may depend upon her having mainly studied these stages in *S. erectum*.

Shortly after the partition of the ES the plasma in the chalazal chamber vacuolizes. Fig. 5B shows a one-nucleate chamber with two big vacuoles. The divisions of the nuclei in the chalazal and micropylar chambers are not synchronous. In the figure one nucleus in the chalazal chamber and two in the micropylar one can be seen. Fig 6A, on the other hand, shows the nucleus of the micropylar chamber just in division while the chalazal chamber has two nuclei, one of which just dividing for the second time. This illustrates that even the nuclei within one chamber sometimes divide asynchronously. Often, the chalazal chamber remains 2-nucleate for some time, while the nuclei in the micropylar chamber continue to divide. Fig. 5C shows four nuclei in the micropylar chamber and only two in the chalazal. Sometimes the nuclei have more than one nucleolus, as illustrated in the same figure. Regarding the divisions, they sometimes occur synchronously. In Fig. 6B four nuclei can be seen in each chamber. As has been shown previously, the divisions in the micropylar chamber

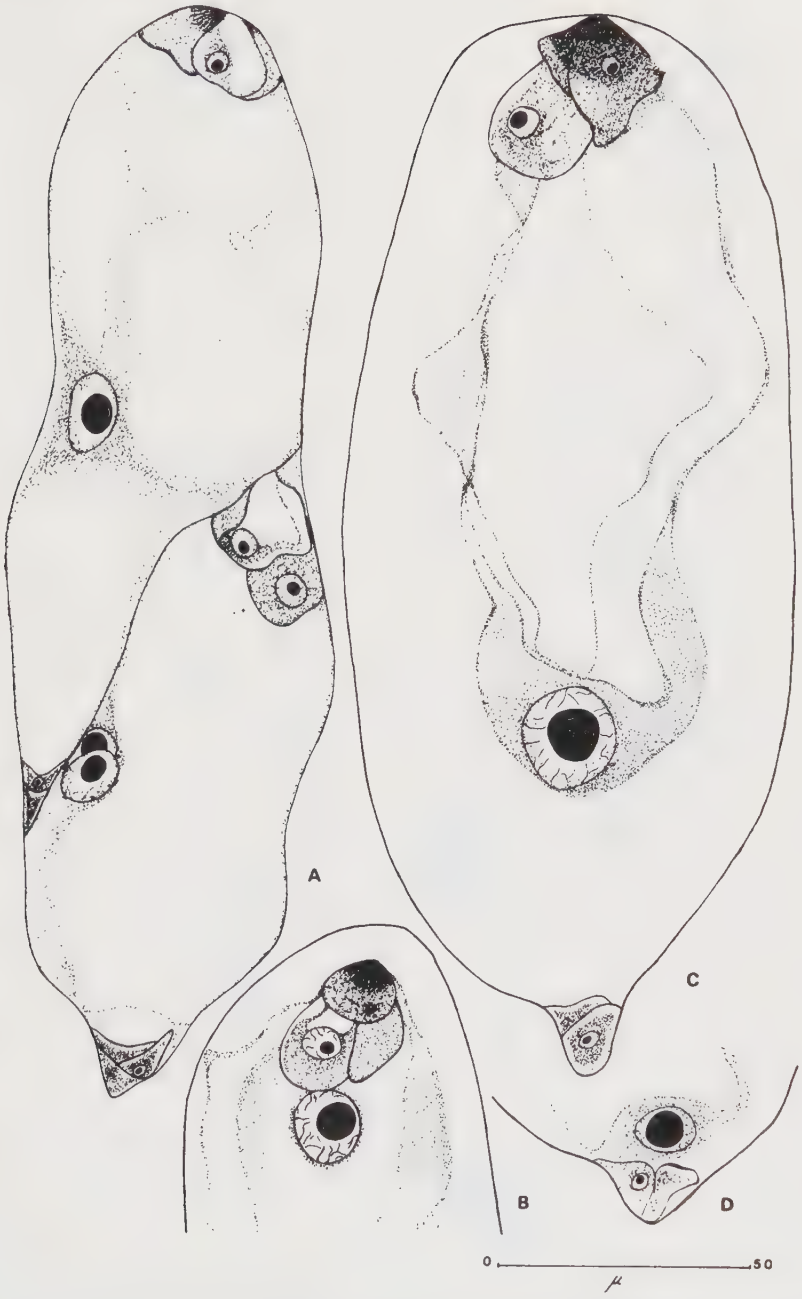

Fig. 4. (A)*Sparganium erectum.* (B) *S. minimum.* (C) *S. simplex.* (D) *S. angustifolium.* (A) Two competing, well developed ES. (B) The fusion nucleus situated close to the egg apparatus. (C) The primary endosperm nucleus located in the lower part of the ES. (D) The primary endosperm nucleus just above the antipodes.

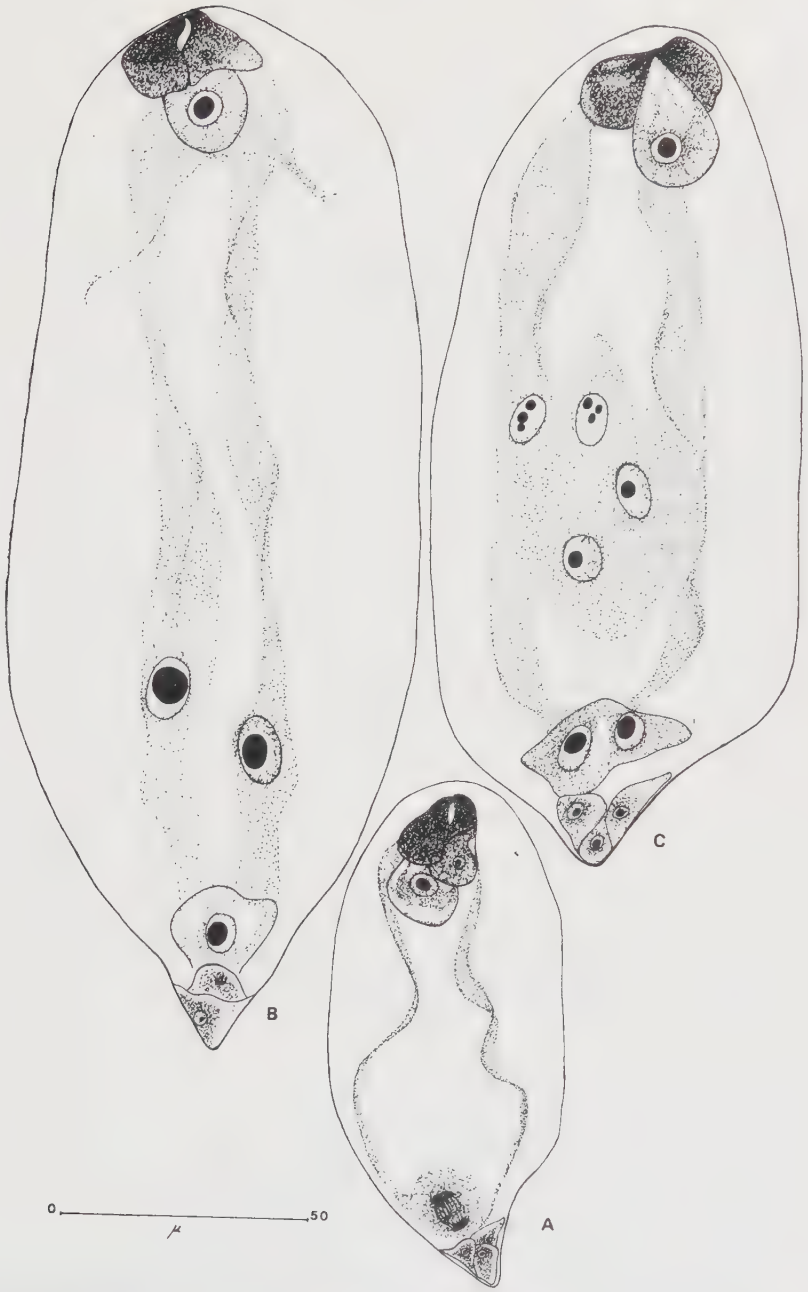

Fig. 5. (A) *Sparganium glomeratum.* (B) *S. angustifolium.* (C) *S. minimum.* (A) The primary endosperm nucleus in division. (B) A one-nucleate chalazal chamber. The micropylar endosperm with two nuclei. (C) Four nuclei in the micropylar chamber, two of which with more than one nucleolus. The basal chamber has two nuclei.

are always free nuclear, forming a nuclear type of endosperm. The first two divisions in the chalazal chamber always seem to be free nuclear, but then wall formations sometimes take place. In Fig. 7A four nuclei can be seen in the micropylar chamber and five one-nucleate cells, with a large vacuole in each, in the chalazal part of the ES. There is no change within the antipodes so far. All three remain in the original condition with a quite distinct nucleus and homogeneous plasma. This interesting wall formation within the chalazal chamber is similar to what I have found in the genus *Typha* (op. cit.). There the basal endosperm remains 2-nucleate for a period of time while the nuclei in the micropylar chamber continue to divide. I have found up to eight nuclei in the micropylar chamber while only two in the chalazal one. The first division is always free nuclear, but then a wall formation may occur. In *Sparganium* I have never found a two-celled chalazal chamber. It seems as if the nuclei divide once more before the wall formations take place. Fig 6C shows six nuclei in the micropylar chamber and four in the chalazal one. In Fig. 7B there are seven nuclei in the micropylar chamber and six in the chalazal one. A synchronous division in the micropylar chamber is illustrated in Fig. 8A where all the eight nuclei are in division. The chalazal chamber has five nuclei. Sometimes the plasma of the basal chamber contains small vacuoles as in Fig. 8A, sometimes there is only one large vacuole. An ES with twelve nuclei in the micropylar chamber and an asynchronous division in the chalazal chamber can be seen in Fig. 9A.

The subsequent development follows two different lines. Either the nuclei in the chalazal chamber divide freely and form a coenocytic cell (Figs. 9B and C) with a great number of nuclei in the plasma, or the divisions will be followed by wall formations and result in a large hemispherical mass of cells (Figs. 8B–E and 10A). The cells are usually first uninucleate, Fig. 7A, but sometimes they have more than one nucleus, Figs. 8B, C and D. Later they become multinucleate as can be seen on Fig. 8E, where only the cells from one section are shown. At this very late stage the cells are very vacuolized.

The micropylar endosperm is first nuclear but later it becomes cellular (Fig. 10A) and its cells like those of the chalazal chamber are first uninucleate and then multinucleate (Fig. 10C). The zygote remains undivided for quite a while. On Fig. 9B can be seen a three-celled embryo and a lot of nuclei in the micropylar endosperm chamber. During the development just described the antipodes retain their original shape and even at such late stages as shown in Figs. 8E, 9B and C hardly any signs of degeneration can be seen, this usually occurring at a very late stage. Thus there is no multiplication of the antipodes within the genus *Sparganium* as has long been considered valid. Again an observation similar to what I have found in *Typha*. Campbell (1899) published two papers about the embryo sac development in *Sparganium simplex*, *S. Greenii*, and *S. eurycarpum*. He observed no early stages, but stated that the development probably followed the *Polygonum* type. Particularly he remarked the small size of the antipodes in the mature ES. After the fertilization he believed he had observed

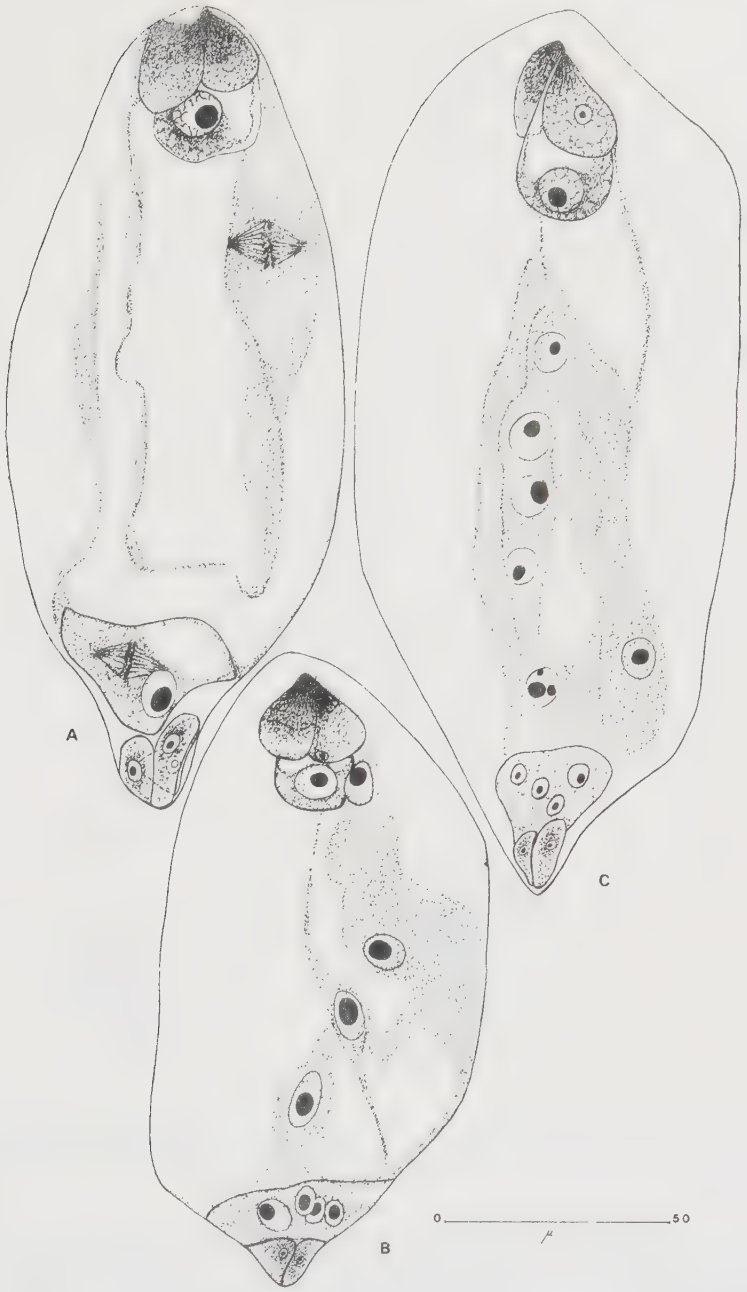

Fig. 6. (A, C) *Sparganium minumum.* (B) *S. erectum.* (A) The nucleus in the micropylar chamber in division. The chalazal chamber with two nuclei one of which in division. (B) Four nuclei in each chamber. (C) Six nuclei in the micropylar and four in the chalazal chamber.

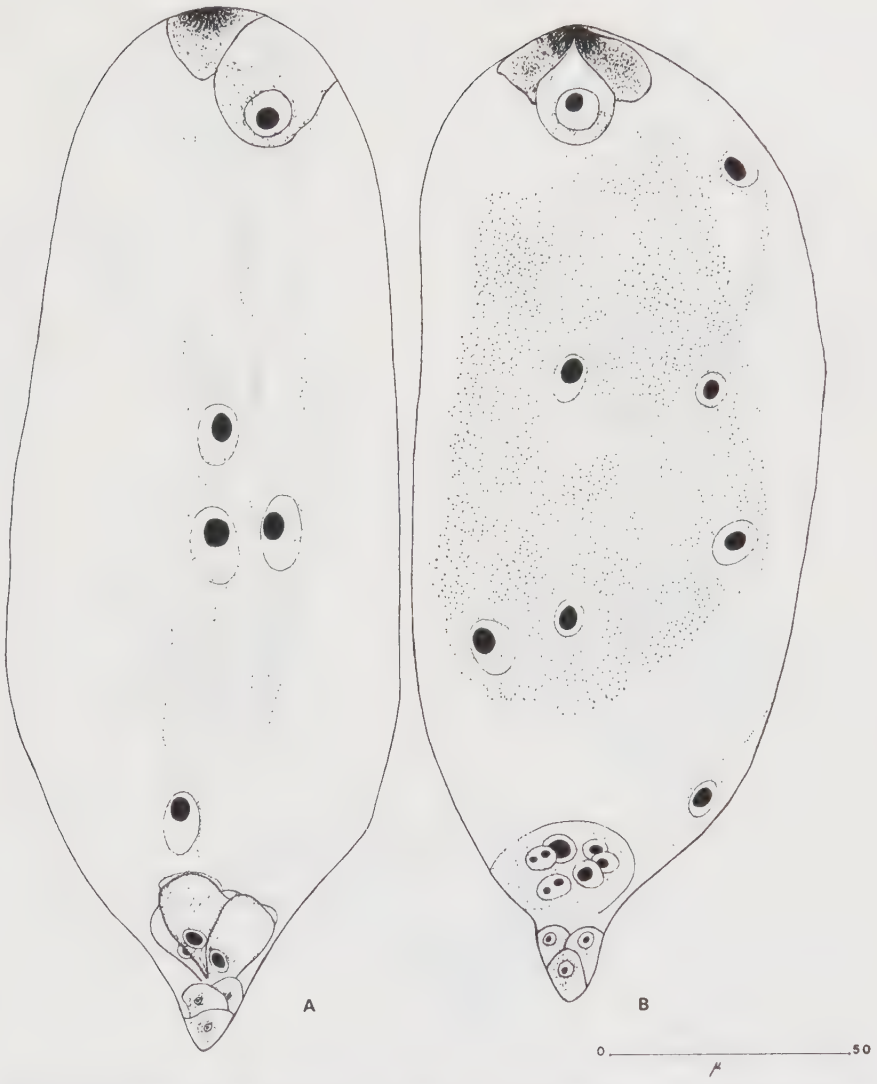

Fig. 7. (A) *Sparganium angustifolium.* (B) *S. simplex.* (A) The chalazal part of an ES with five one-nucleate cells. (B) The micropylar chamber with seven nuclei. The chalazal chamber 6-nucleate.

that the antipodes enlarged to several times their original dimensions and that they presented all the appearances of actively growing cells. He reported the first division of the endosperm nucleus to take place at about the same time as the first nuclear division occurred in the antipodal cells. Before any cell division takes place in the endosperm, the antipodal cells should have increased enormously in size and divided until they form a conspicuous hemispherical cell-mass, each cell clearly defined and

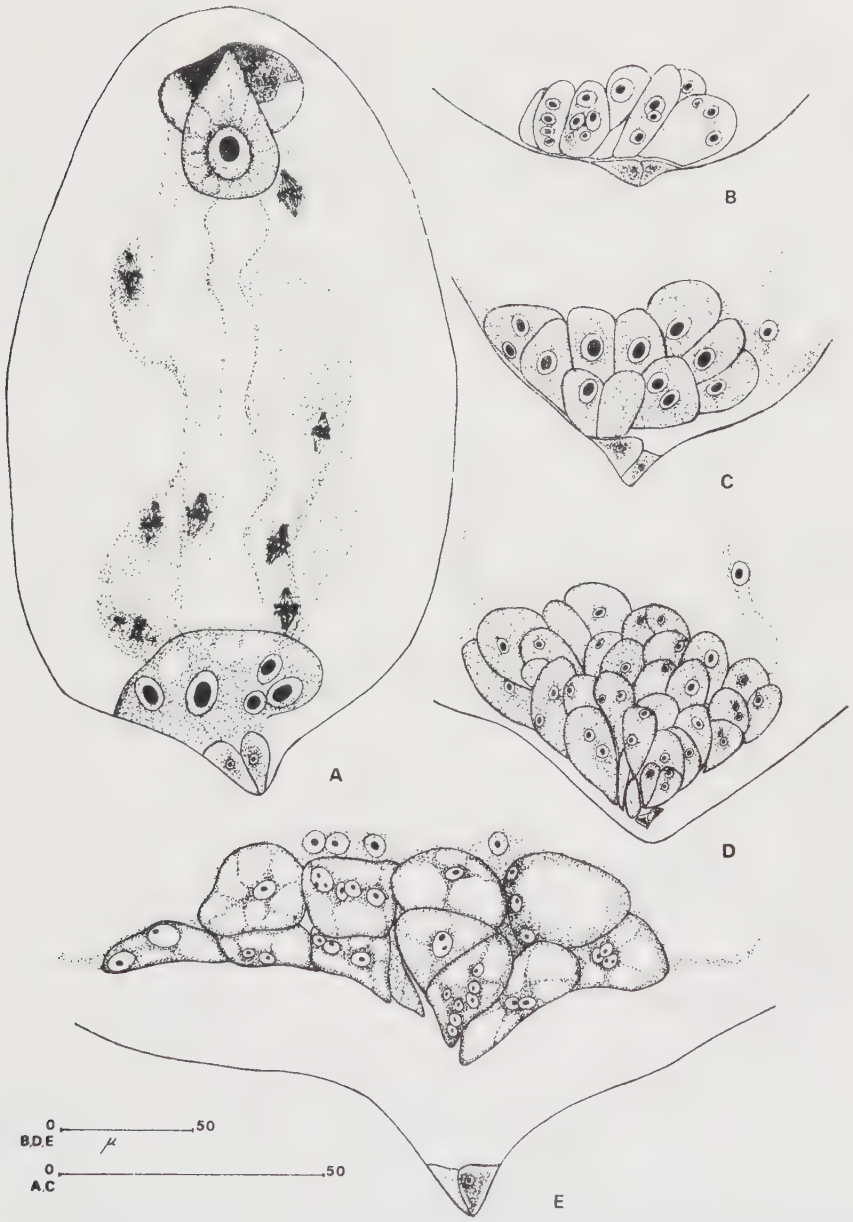

Fig. 8. **(A, B)** ***Sparganium glomeratum.*** (C) *S. simplex.* (D) *S. angustifolium.* (E) *S. erectum.* **(A) A synchronous division in the micropylar chamber. Five nuclei in the chalazal one. (B) The chalazal part of an ES with multinucleate cells. (C) Cellular chalazal endosperm. Some cells have two nuclei. (D) A large hemispherical mass of cells, some two-nucleate. (E) Later stage with many cells some of which showing on the figure. The cells are multinucleate and vacuolized. As in B–D the antipodes are still showing.**

uninucleate. He further thought that the group of antipodal cells functioned as an endosperm in the early stages of development.

In 1912 Tischler expressed his doubts regarding the multiplication of the antipodes. He noticed a similarity to the Helobial type of endosperm and Palm (1915) expressed similar thoughts. Schürhoff (1920) made some research on "verschiedenen *Sparganium*-arten", and stated he "konnte die Angaben Campbells voll und ganz bestätigen". However, his investigations seem to be quite inadequate. The figure he cites as an evidence shows the basal part of an ES with a large primary endosperm nucleus and two antipodes with signs of degeneration. He misinterprets the antipodes as dividing mitotically.

When studying Campbell's figures (1899*b*: 326, plate 47) it is surprising how he could so completely misunderstand what he evidently saw. There are many figures showing two antipodes and above them a chalazal endosperm chamber, which he interprets as the third antipod suddenly becoming an actively growing cell. Naturally he did not know anything about the Helobial type of endosperm as that was not known at that time. What is remarkable, however, is that he never observed the real third antipod, which in most cases must have been visible in one of the neighbouring sections.

U. Müller-Doblies's (1969) description of the endosperm development is almost fully in agreement with my own observations reported above and in my paper of 1968. She states that the divisions in the micropylar chamber are mostly synchronous and thus in number a multiple of two, 2^n. She gives only a few exceptions to this statement. According to my own observations I have for all the examined species found that the main part of the nuclei divide synchronously but the rest do not. These exceptions are far too many to find any rule such as 2^n valid. In accordance with my own observations U. Müller-Doblies describes the development in the chalazal chamber to be either nuclear or cellular. She has counted the number of cells to be about 400 and found them to be multinucleate even at quite young stages. In *Typha* I have found around twenty cells in the cellular line of development of the chalazal chamber, but never more than ten nuclei in the coenocytic one. In *Sparganium* there are many more. The cells formed by the chalazal chamber can amount to 300–400 at late stages, and within a coenocytic chamber in *S. simplex* (Fig. 9C) 59 nuclei were counted. Even at quite early stages the cells may have more than one nucleus (Figs. 8B, C and D), but not as many nuclei as is frequent at late stages (Fig. 8E).

In the summary of the variation of the Helobial type of endosperm given by Swamy & Parameswaran (1962, p. 29) they stated for the monocotyledons among other things: (*a*) the endosperm in the micropylar chamber is first nuclear and later becomes cellular; (*b*) if the nucleus in the chalazal chamber divides the division occurs freely; (*c*) if a coenocytic chalazal chamber is formed the first few divisions in the micropylar and chalazal chambers are synchronous; and (*d*) if in the chalazal chamber a wall formation occurs it is significantly belated not only in its own ontogeny but also as

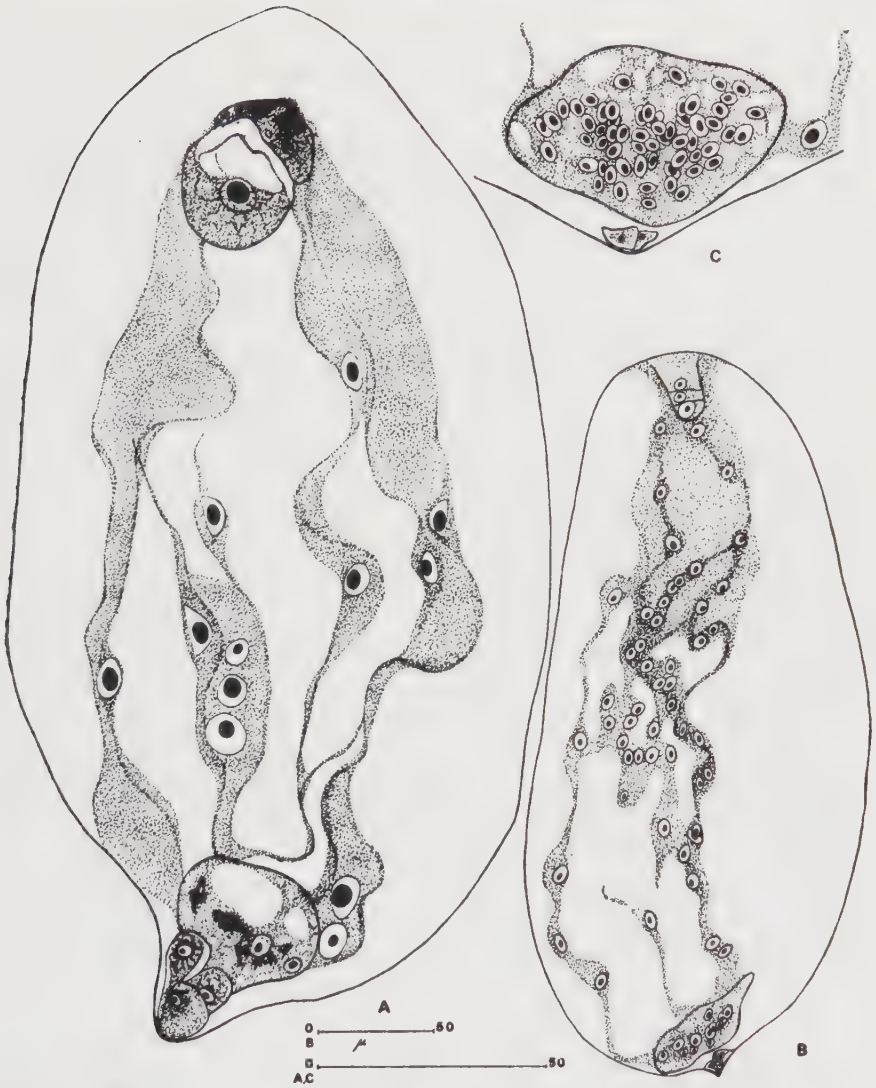

Fig. 9. **(A)** ***Sparganium minimum.*** **(B, C)** ***S. simplex.*** **(A) The nuclei of the chalazal chamber dividing asynchronously. The micropylar chamber with several nuclei. (B) ES with a great number of nuclei in the micropylar chamber. The embryo is three-celled and the chalazal chamber a coenocytic cell. (C) A large coenocytic cell with 59 nuclei.**

compared with the time of initiation of cells in the micropylar chamber. For *Sparganium* like *Typha* the first statement is correct. Regarding the second it is true for the first two divisions (in *Typha* only for the first), but then only for one of the above-mentioned lines of development in the chalazal chamber. Like *Typha* there is no synchronous division as mentioned in the third statement and finally the first wall formation is delayed, but not in comparison with the conditions in the micropylar chamber.

As mentioned in my previous paper Maheshwari (1950) discusses the variability in the Helobial type of endosperm. Among the examples he gives where the division in the chalazal chamber is accompanied by wall formations is a report by Juel (1907) according to *Saxifraga granulata*.

The first few divisions in the chalazal chamber may be free nuclear with wall formations occurring later on. Maheshwari regards this type of development as quite unusual, and mentions among the cases first reported *Heloniopsis breviscapa* (Ono 1927).

Swamy and Parameswaran are of the opinion that the typical Helobial type of endosperm is to be found only in the monocotyledons. The reported occurrence of a Helobial development of endosperm in the dicotyledons they have found to be only a modification of the Cellular or Nuclear types. The greatest similarity to the monocotyledonous Helobial endosperm was found in some genera of the families *Grossulariaceae* and *Saxifragaceae* and in the genus *Lycopsis* of the *Boraginaceae*. However, "it should be emphasized that such similarities are restricted to only specific stages of ontogeny and the ontogenies themselves in their entirety are by no means comparable" (op. cit. p. 44). In a summary of the most important differences between the Helobial endosperm of the monocotyledons and that reported in the dicotyledons they stated that in the monocotyledons the cell formation in the chalazal chamber succeeds that in the micropylar chamber, while in the dicotyledons cell formation precedes in the chalazal chamber. They had not found any exception from this rule.

Hamann (1966) presented the embryological conditions in *Philydraceae*. He reported cell formation to take place earlier in the chalazal chamber than in the micropylar one. Thus a development of the Helobial endosperm very much alike the conditions within the *Typhaceae* and the *Sparganiaceae*. The three above mentioned families are all exceptions from the rule stated by Swamy and Parameswaran. On the other hand, they are not the only known exceptions. In *Bromeliaceae* a similar type of development has been found in *Lindmannia penduliflora* by Lakshmanan (1967). With these exceptions mentioned, I can not find the rule valid.

In the examined species of *Sparganium* I have found the first two divisions in the chalazal chamber to be free nuclear ones. Then very frequently wall formations take place. Like U. Müller-Doblies I have never found a chalazal chamber with only two cells. The following development follows two different courses: one forming a

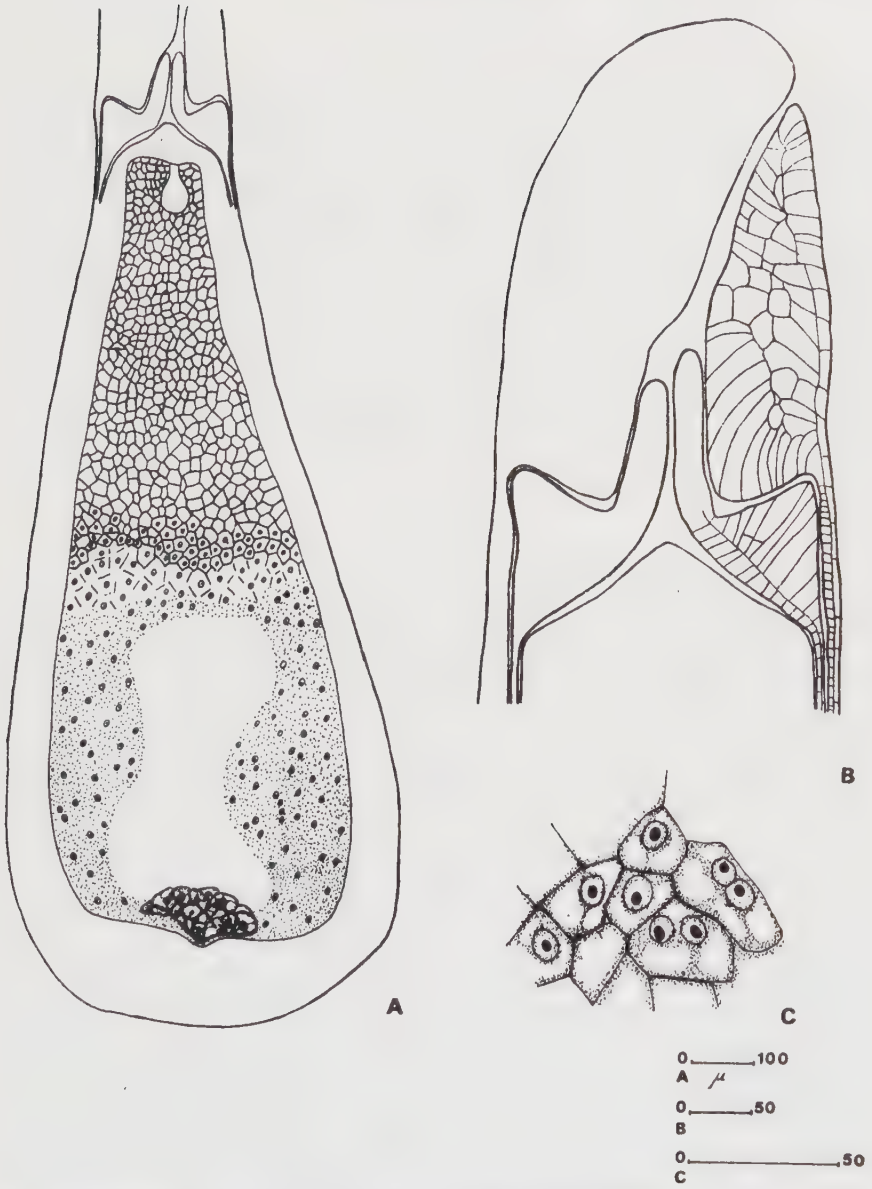

Fig. 10. Sparganium erectum. (A) The micropylar endosperm is just becoming cellular. The chalazal endosperm with many cells. (B) The integuments forming a "Samendeckel". (C) Cells of the micropylar endosperm at late stage. They are vacuolized and some two-nucleate.

large hemispherical mass of cells, the other forming a coenocytic cell with a great number of nuclei in the plasma. I have found about 300–400 cells at late stages in the cellular chalazal part of the ES, but not more than 59 nuclei in the coenocytic chalazal chamber. According to my observations I will draw the conclusion that the cellular course of development exists in a higher degree than the nuclear one. There must be many intermediate cases where wall formations occur after several free nuclear divisions. In *Typha* I found them to exist in about the same degree. The cells are first uninucleate, but soon they become multinucleate. At late stages their plasma is vacuolized. At the beginning the nuclei in the coenocytic chalazal chamber are large and distinct but at late stages they become smaller and start to disintegrate. The micropylar endosperm is first nuclear and later it becomes cellular with several multinucleate cells.

Discussion

Since Campbell (1899) reported that a multiplication of the antipodes exists within the genus *Sparganium* and Schürhoff (1920) confirmed his statement there has been some speculation whether the same thing occurred within the genus *Typha* or not. Schoenebeck, a pupil of Schürhoff, published in 1924 a report with most doubtful figures, and confirmed the expectation according to multiplication of the antipodes. In 1968 I indicated that *Typha* like *Sparganium* has a Helobial type of endosperm, and after further investigations (1972) I described the embryological conditions within *Typha*. With the report by U. Müller-Doblies (1969) and the present one also the embryology within *Sparganium* is known. Campbell, Schürhoff, and Schoenebeck were all wrong regarding the multiplication of the antipodes. *Sparganium* and *Typha* have a Helobial type of endosperm and lack antipodal multiplication.

The above described embryological development in the genus *Sparganium*, both regarding the microsporogenesis and male gametogenesis and the macrosporogenesis and female gametogenesis, shows a great similarity to the one described for *Typha*. There are only a few small differences between the genera. Important characters such as the development of the tapetum, the microspores, the antipodes, and the endosperm are entirely the same. Noticeable is the high correspondence in characters such as the endosperm development within the chalazal chamber and the mixture of different microspore tetrad forms, characters that are regarded unusual.

These embryological conditions conclusively underline the close relationship between the genera in question. Within the genus *Sparganium* there is a great accordance in the development between the different species. The main difference is the size of the ES. Regarding the embryology within *Pandanaceae*, the third family assigned to the order *Pandanales*, this is only known for *Pandanus* and *Freycinetia*, two of the three genera usually included in the family. The embryological conditions within *Sararanga* are still unknown. The ES of *Pandanus* was described by Campbell (1909, 1910 and 1911) and reinvestigated by Fagerlind 1940. The last named author also

described some stages of the ES development in *Freycinetia*. His pupil Strömberg (1956) made a more complete examination of the latter genus. Referring to my previous paper there are embryological features that support a connection between *Pandanus* and *Freycinetia*, such as the construction of the integuments, the development of the nucellar epidermis, and the hypostasis. Both genera have an anatropous, bitegmic, and crassinucellar ovule. The ES development follows the *Polygonum* type and the endosperm formation is Nuclear in both genera. In *Pandanus* the micropyle is formed by the inner integument only, whereas in *Freycinetia* both integuments are involved. In some species of *Pandanus* Fagerlind observed an increase in number of antipodal cells through a migration of nuclei from the somatic neighbouring cells. Strömberg reported no antipodal multiplication in *Freycinetia*. There the antipodes degenerate very early.

The above mentioned facts show that there are only some few characters that are the same for the *Typhaceae* and the *Sparganiaceae* on the one hand and the *Pandanaceae* on the other. The development of the antipodes and the endosperm are entirely different. No comparison is possible with respect to the pollen development as this is not yet fully investigated for the *Pandanaceae*. The chromosome number for *Pandanus* is reported as $n = 30$ and $n = 32$ by Harada (1947) and Tjio (1948). For *Sparganium* is reported $n = 15$ (Löve A. & Löve D. 1948), and the same number is found for *Typha* by among others Roscoe (1927), Clausen (1927), and Harada (1947).

As mentioned above, the morphology of the pollen grains has been described by Erdtman (1952). He found the grains in the *Pandanaceae* to be different from those of *Sparganium* and *Typha*, and recommends the *Sparganiaceae* to be referred to as a subfamily, *Sparganioideae* under *Typhaceae*.

The facts reported by Erdtman and illustrated by my scanning pictures further corroborate the high relation between the *Sparganiaceae* and *Typhaceae* and the disrelation between those and the *Pandanaceae*.

As the result of a serological investigation Lee & Fairbrothers (1972) made the following conclusions: a significantly high serological correspondence between *Typha* and *Sparganium*, supporting the placement of the two genera within the order *Typhales*; low serological correspondence between the *Typhales* and *Liliales*, indicating possible distant evolutionary relationship between these two orders; and little or no serological correspondence between the *Typhales* and most of the tested monocotyledons, including *Pandanus*, reflecting an isolated position for the *Typhales* within the monocotyledons.

Regarding the first statement the high serological correspondence is still a character that underlines the close relationship between *Typha* and *Sparganium*. According to the two last mentioned statements still many investigations of important taxonomic characteristics are needed before commenting whether these indications are correct or not.

D. Müller-Doblies (1969) presented the result of a comparable investigation with

respect to the morphology of the inflorescences and flowers of *Sparganium* and *Typha*. In the summary concerning the taxonomy he says: "Unterschiede, Gemeinsamkeiten und Progressionen im Infloreszenz- und Blütenbereich werden für beide Gattungen zusammengestellt (S. 516–518). Von den fünf unterscheidenden Merkmalen, mit denen Engler die Aufstellung der *Sparganiaceae* begründete und ihre Anerkennung durchsetzte, sind drei falsch und zwei wertlos (S. 521). Die wenigen verbleibenden, allerdings z. T. augenfälligen Unterschiede lassen sich weitgehend als Anpassung der Gattung *Typha* an die Anemochorie verstehen. Sie liegen weit unter einer Mindestforderung für getrennte Familien. Die Familie *Sparganiaceae* wird eingezogen, und die *Typhaceae* s.l. werden beschrieben." The result obviously points to the close relationship between the genera in question.

According to the above described disrelation regarding the morphology, the palynology and the embryology within *Pandanales* between the *Pandanaceae* on the one hand and the *Sparganiaceae* and the *Typhaceae* on the other the two last mentioned families should be removed from *Pandanales* and put into an order of their own, the *Typhales*, forming one single family, the *Typhaceae* with two subfamilies, the *Typhoideae* and the *Sparganioideae*.

Acknowledgement

I am much indebted to Professor Gunnar Harling for valuable discussions and help.

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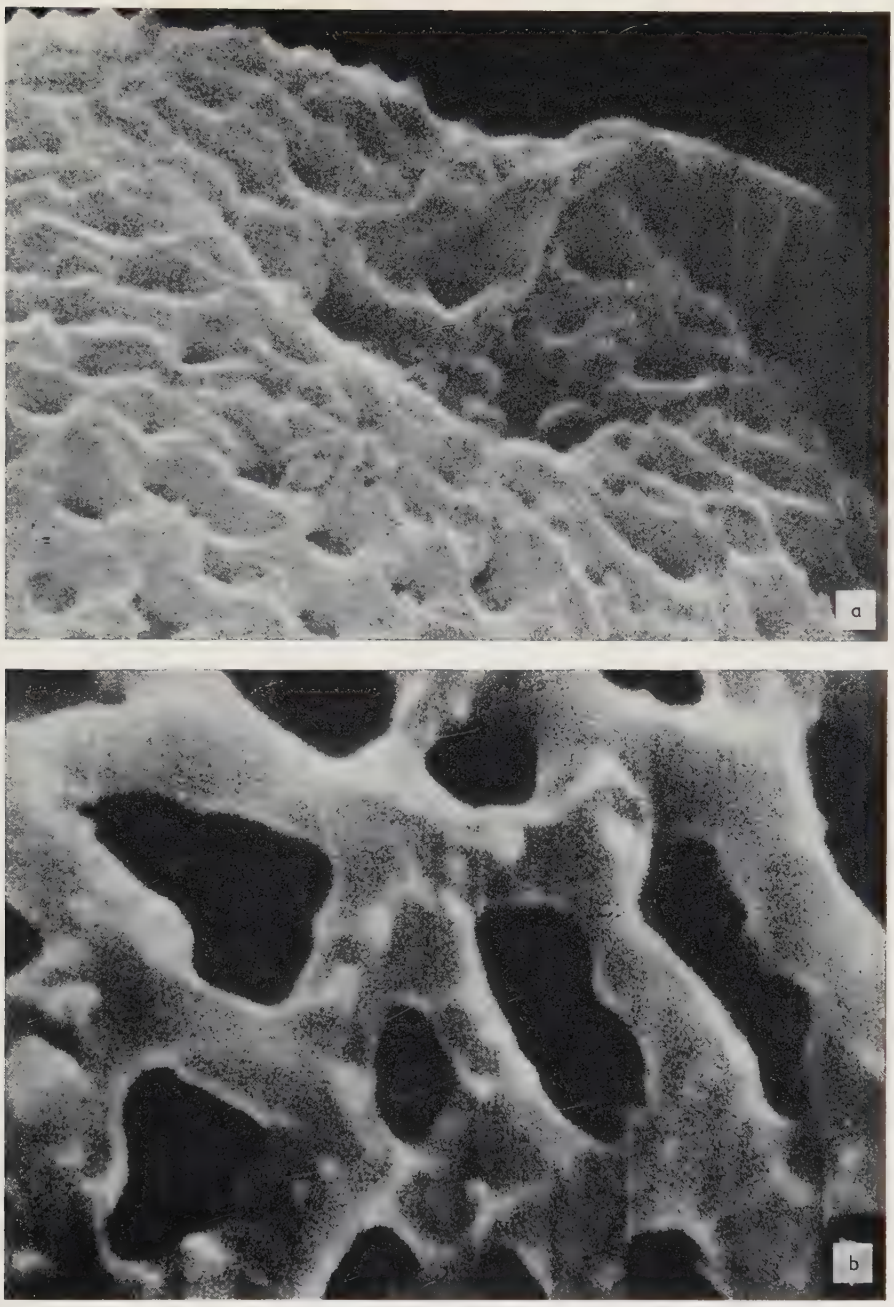


Plate I. (a) Sparganium erectum. (b) S. simplex. (a) A pollen grain of spheroidal shape with ulcus showing. ×29 000. *(b)* A detail of the wall surface with reticulate pattern. ×56 200.

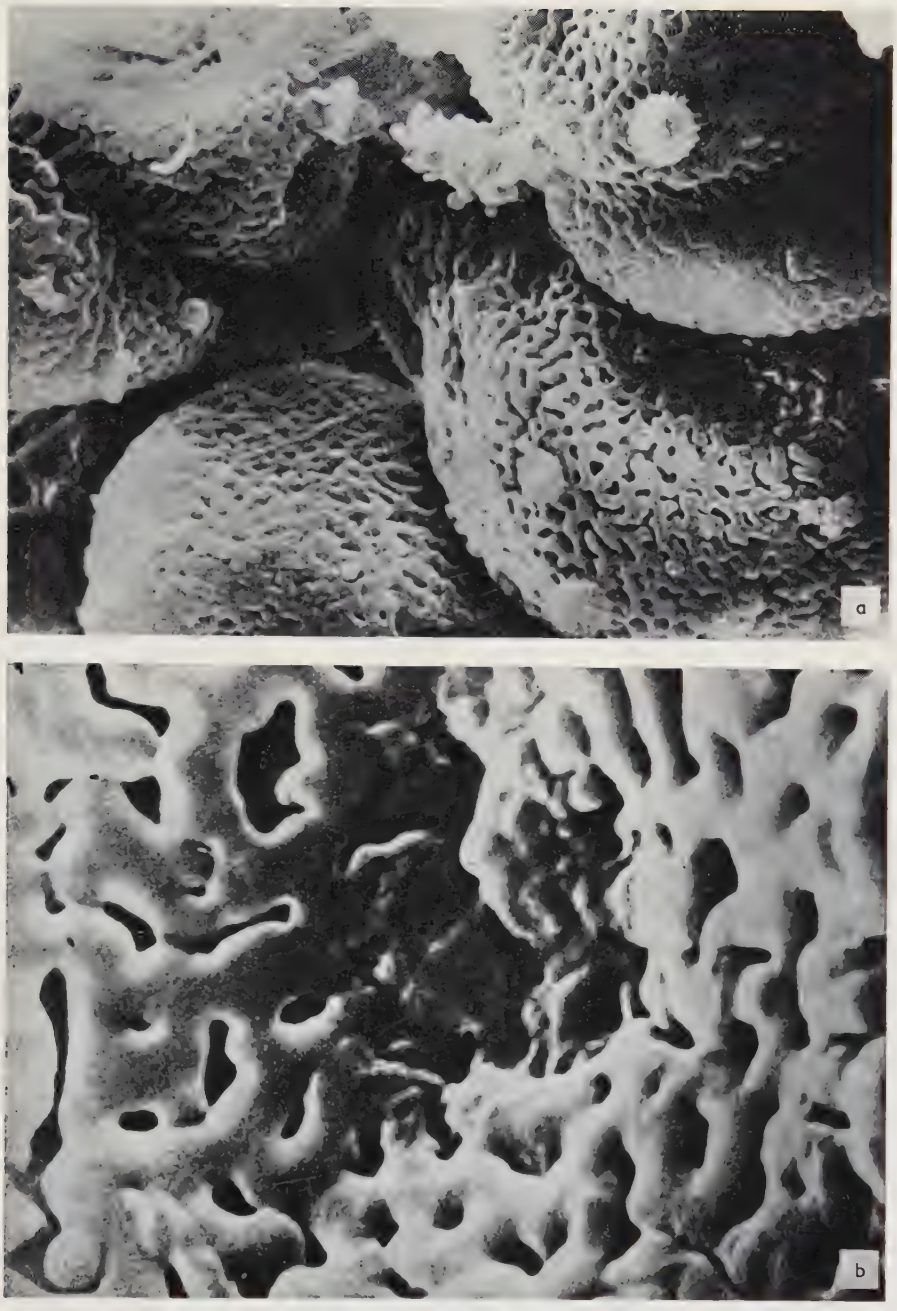
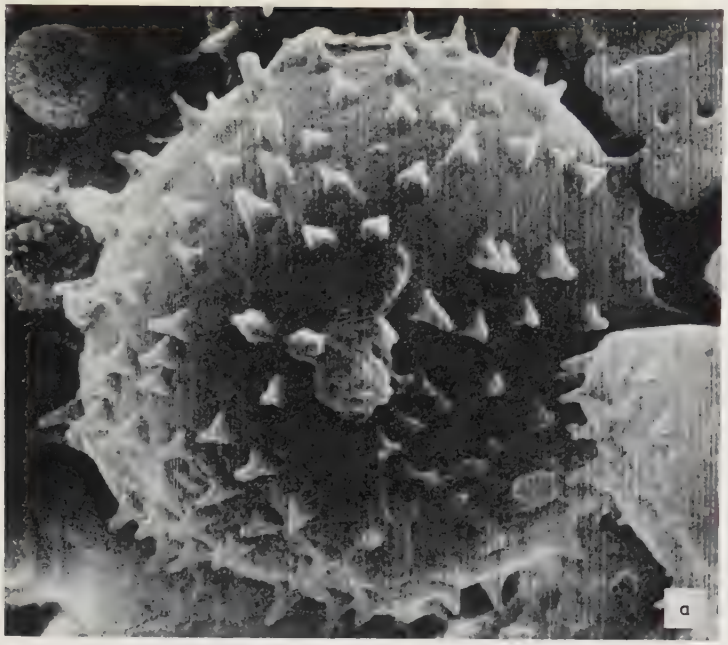


Plate II. Typha latifolia. (a) Some spheroidal pollen grains with one ulcus showing. On the picture can also be seen several spores from some fungus. ×5 800. *(b)* The ulcus with the reticulate pattern of the wall surface. ×40 300.



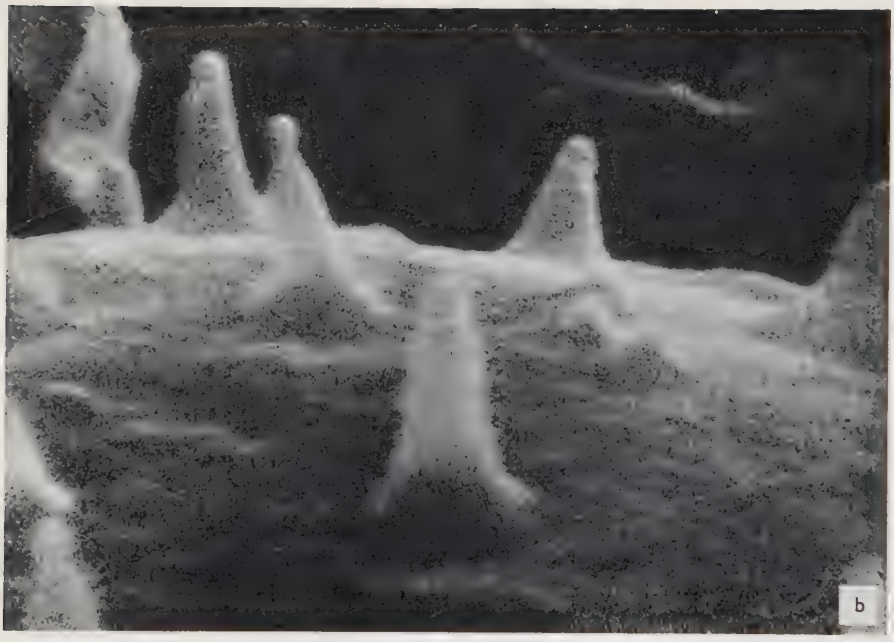


Plate III. Pandanus fascicularis. (a) A pollen grain of more or less spheroidal shape. ×11 700. *(b)* The wall surface is spinuliferous. ×48 700.

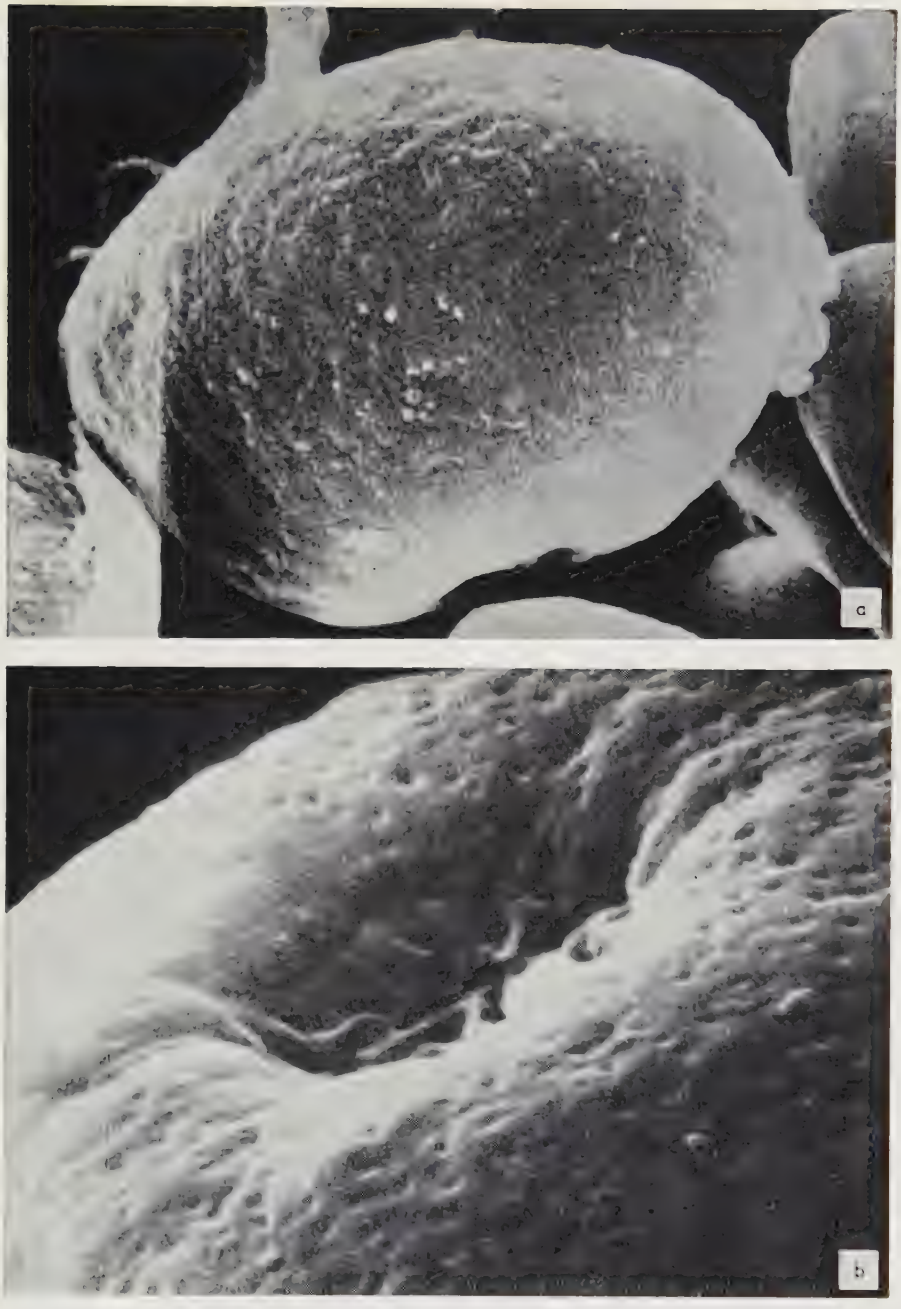


Plate IV. Freycinetia Banksii. (a) A pollen grain of ellipsoidal shape. ×10 200. *(b)* The psilate wall surface with the ulceroid—faintly sulcoid aperture. ×42 900.

Embryological Studies in Helenieae-Heleninae (Compositae)

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ABSTRACT

Five genera in the Compositae-Helenieae-Heleninae have been examined: *Chaenactis* DC., *Othake* Raf., *Palafoxia* Lag., *Monolopia* DC. and *Cadiscus* E. Mey. Microsporogenesis and male gametogenesis follow, with only minor deviations, the normal pattern of development found in the family. The mature pollen grains are three-celled, triporate and more or less spheroidal, with a spinuliferous exine. Macrosporogenesis and female gametogenesis are as follows: in all species studied the archespore is one-celled, excepting *Chaenactis glabriuscula*, in which a two-celled archespore has also been found. In the genus *Chaenactis* the ES development is monosporic in *Ch. glabriuscula* and *Ch. Fremontii*. In *Ch. artemisifolia*, however, the ES development was bisporic in about 90% of the material studied and tetrasporic in about 10%. Three antipodes were found in *Ch. artemisifolia*, two in *Ch. glabriuscula*, and two, three or five in *Ch. Fremontii*. The genera *Othake* and *Palafoxia* showed a tetrasporic ES development, except in about 20% of the material of *Othake texanum* studied, in which the ES development was monosporic. The number of antipodes found in *O. texanum* is three to six, in *O. sphacelatum* one to six and in *P. linearis* three or four. *Monolopia gracilens* has a monosporic ES development and two or three antipodes. In *Cadiscus aquaticus* ES development is monosporic and the number of antipodes three or four, of which the lower one alone occupies 1/3 of the entire space of the ES.

The results of this embryological study support Rydberg's (1914) subdivision of the annual species of the genus *Chaenactis* into the two sections Acarphaea and Euchaenactis. Furthermore, the embryology reveals a close relationship between the genera *Othake* and *Palafoxia*, which supports the proposal by Turner & Morris (1976) to recognize only a single genus, *Palafoxia*. Embryologically the genus *Cadiscus* shows a close relationship with the Senecioneae - Othonninae.

INTRODUCTION

Many embryological studies have been made within the Compositae, although very unequally distributed among the different tribes. Some tribes are rather well investigated while about others very little is known. To my knowledge *Gaillardia* (Rosén 1944) is the only genus so far examined within the Helenieae - Heleninae. Further studies are thus required within this group. The ES development of the majority of species within the Compositae (Davis 1966) follows the Polygonum (Normal) type, though the Allium type has also been reported in a few cases. Several variations of tetrasporic development have been described, especially in the Astereae and Anthemideae (Harling 1950, 1951a, b). The in this family very unusual Fritillaria type has so far been found only in the genera *Rudbeckia* and *Gaillardia*. Since *Gaillardia* belongs to the tribe Helenieae an investigation of other genera in the same tribe would be of interest.

There has been a lot of discussion about the validity of the Helenieae as a tribe. Bentham (1873 p. 381) declared that "our Helenioideae" was not a distinct tribe, unlike most of the others, "for the genera we have grouped under that name pass respectively into Helianthoideae, from which they differ chiefly in the absence of paleae to the receptacle, or into Anthemideae, from which they are separated by the involucre, habit, and in great measure by geographical distribution; and some species or small genera have almost the pappus, as well as other characters, of Senecionideae". Nevertheless, in uniting this group of genera he thereby left the remaining tribes much better defined.

The subtribe Heleninae (Hoffmann 1894) comprises an extremely diverse group of genera virtually representing the residue from the segregation of the other subtribes. Rydberg (1914) arranged the genera into smaller groups. From the results of a study of chromosome numbers in the tribe Helenieae, Raven & Kyhos (1961 p. 849) stated that "it seems exceedingly doubtful that all of these diverse groups are closely related". However, they suggested that in view of the essential unity in distribution

and habit, it may be convenient to retain them as a tribe, at least until further studies provide the necessary information for their rearrangement. Other authors, such as Turner (1956) and Carlquist (1959), have expressed similar views.

It is hoped that the following embryological study provides some useful information for the taxonomical discussion.

MATERIAL AND METHODS

The following genera were examined: *Chaenactis* DC., *Othake* Raf., *Palafoxia* Lag., *Monolopia* DC. and *Cadiscus* E. Mey. The material mainly originates from plants cultivated in Uppsala Botanical Garden, the Hortus Bergianus in Stockholm and the Botanical Garden in Göteborg from achenes received from U.S.A. Some fixed material was supplied by Dr Peter H. Raven, St. Louis, Missouri, U.S.A. and Dr A.V. Hall, Cape Town, South Africa. The main part of the material was fixed in Karpeschenko´s fixative, but in a few instances FAA was used. Some of the material was pre-fixed in Carnoy´s solution. Most of the fixed material was placed in a vacuum chamber, to remove as much air as possible. After dehydration and infiltration by the TBA method, material was embedded in paraffin wax, following Johansen (1940). Sections, thickness 8—14 µm, were then cut on a rotary microtome and stained with Fast Green-Safranin-Gential Violet.

All the microscope slides studied are deposited at the Department of Systematic Botany, University of Göteborg and the voucher specimens are kept at the Botanical Museum, University of Göteborg.

GENUS CHAENACTIS DC.

In the genus *Chaenactis* 33 species have so far been described. The distributional area covers the western United States, centred in California. The embryology of the following species was studied: *Chaenactis artemisifolia* A. Gray, *Ch. glabriuscula* DC., and *Ch. Fremontii* A. Gray. The material of *Ch. artemisifolia* was grown both in the Uppsala Botanical Garden in 1962, from achenes obtained from the Rancho Santa Ana Botanic Garden, Claremont, California, and in the Botanical Garden of Göteborg in 1972 and 1973, from achenes from Uppsala. *Ch. glabriuscula* was also grown at Uppsala in 1962 from achenes obtained from the Rancho Santa Ana Botanic Garden. In addition some fixed material of *Ch. Fremontii* (herbarium number 17114) was sent by Dr. Peter H. Raven.

A. Microsporangium, Microsporogenesis and Male Gametophyte (Fig. 1).

Microsporogenesis and male gametogenesis followed the same development in all the species examined, which are therefore treated together. All the drawings were made from *Ch. artemisifolia* material.

Four different cell layers are discernible in the sporangium wall after the differentiation of the sporogenous tissue: the epidermis, the endothecium, a single middle layer and the tapetum. As in most other Dicotyledons microspore formation is of the simultaneous type. The rounding off of the pollen mother cells (PMC) and their mutual discharge start at about the same time as the meiotic divisions begin, but seems to be a rather slow process. Even during the anaphase and telophase stages of the heterotypic division the cells still show a tendency to remain together in longitudinal rows (Fig. 1 C). This is probably the reason why most of the metaphase and anaphase spindles are orientated more or less longitudinally to the axis of the anthers. No common orientation of the spindles is found during the homotypic

division (Fig. 1 D). Pollen-tetrads of the tetrahedral type are formed. The young tetrad cells are held together by callose capsules (Fig. 1 E, F).

During the early PMC stage (Fig. 1 A) the tapetal cells are always uninucleate. However, it frequently happens that the nuclei have more than one nucleolus. The plasma is dense and appears to be quite homogeneous. Shortly after the start of meiotic division most of the tapetal cells are binucleate (Fig. 1 B). The tapetal cells and their nuclei thereafter increase in size (Fig. 1 C), and their nuclei regularly contain several nucleoli. In the uninucleate cells the nucleus is much larger than the nuclei of binucleate cells (Fig. 1 D, E). At this stage most of the tapetal cells have grown considerably in size and only remnants of the middle layer of the sporangium wall can be seen. The tapetal cells are not uniform. In Fig. 1 F one tapetal cell shows two nuclei dividing. A neighbouring cell, also in course of division, has only a single, but very broad, metaphase plate. As a great many chromosomes are involved it is probable that this nucleus has a higher ploidy than do those of the above mentioned cell.

When the callose capsules have disappeared and the young pollen cells are completely separated from one another, the inner walls of the tapetal cells have already disintegrated. Their cytoplasm has infiltrated between the pollen cells. Most of the nuclei are located close to the wall, but later become distributed between the microspores. A periplasmodium has thus been formed (Fig. 1 G).

During the rather late stage when the uninucleate pollen cells have been acquired an exine the periplasmodium still seems to be vital, but it becomes absorbed completely a little later, before the first mitotic division in the pollen cell (Fig. 1 H). The ripe pollen grain is three--celled, more or less spheroidal and seems to be triporate. The exine of the pollen grains bears very prominent spinuli (Fig. 1 I).

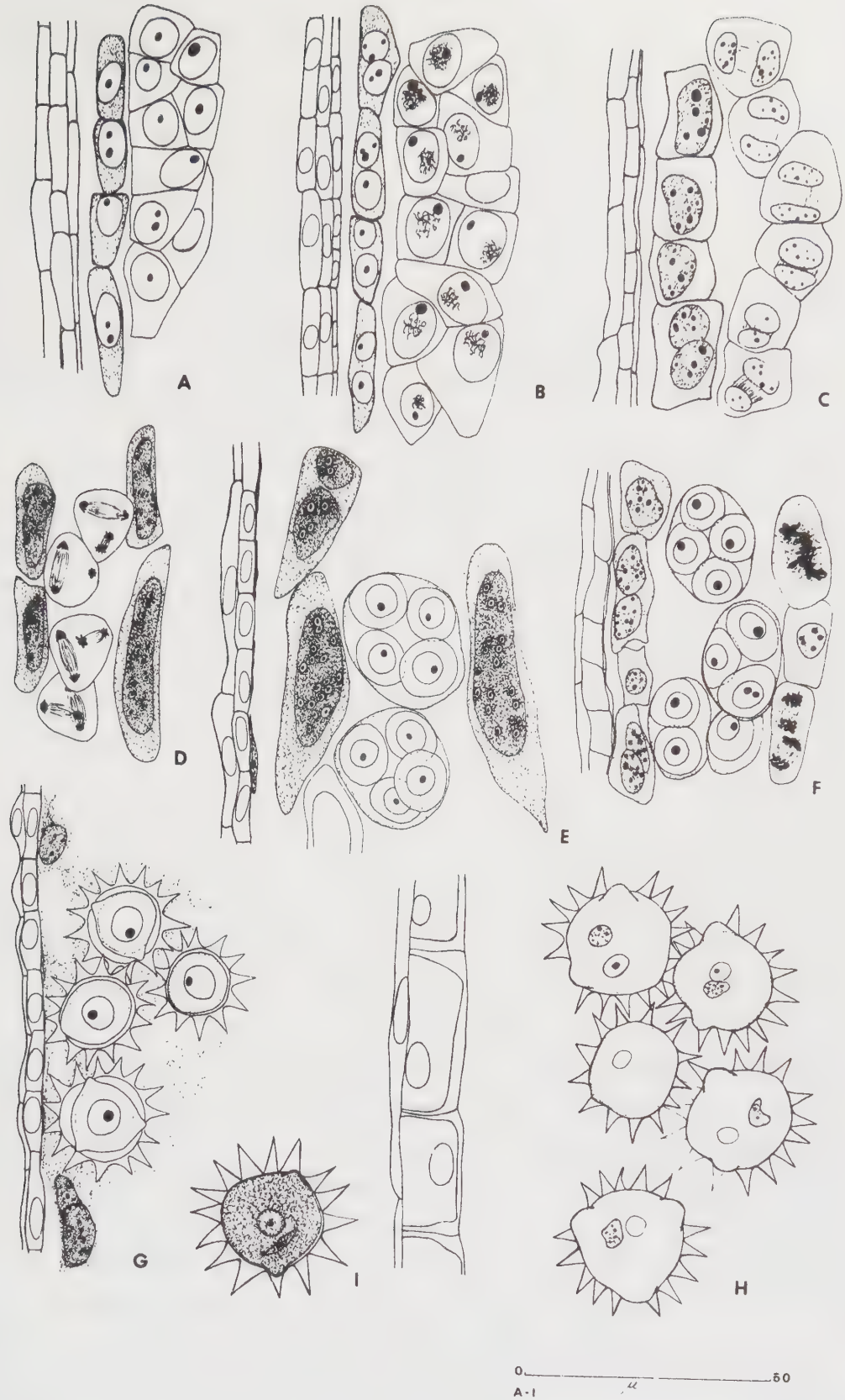

Fig. 1 Microsporogenesis and male gametogenesis in Chaenactis artemisifolia. The sections of the anthers are longitudinal. A. Young anther with uninucleate tapetal cells. The PMC in close contact with one another. B. Later stage. The tapetal cells binucleate. C. The PMC in telophase of the heterotypic division. The tapetal cells either uni- or binucleate, the nuclei large with several nucleoli. D. Anaphase of the homotypic division. Some tapetal cells with one very large nucleus. E. Pollen tetrads. The tapetal cells either uni- or binucleate. Only remnants of the middle layer can be seen. F. The same stage. In two of the tapetal cells the nuclei are in division. G. Uninucleate pollen grains. A periplasmodium is formed. H. Almost mature binucleate pollen grains. The wall surface is spinuliferous. Endothecium with wall thickenings. I. Three-celled mature pollen grain.

B. Macrosporogenesis and Female Gametophyte.

1. *Chaenactis artemisifolia* A. Gray (Figs. 2, 3).

The archespore is one-celled (Fig. 2 B). After enlargement and elongation the embryo sac mother cell (EMC) divides meiotically (Fig. 2 B—E). Towards the end of the heterotypic division a more or less centrally--placed cell plate is formed (Fig. 2 D). In about 90% of all cases seen this is replaced by a regular cell wall, resulting in two dyad cells of which the apical one is smaller than the basal one. The nuclei of these two cells then divide without the formation of cell walls, giving rise to two bivalent coenomacrospores (CMS) (Fig. 2 E). The apical one soon degenerates without showing any sign of enlargement or vacuolation (Fig. 2 F—I). The basal CMS almost immediately enlarges and becomes vacuolate, an indication that the CMS has now developed into a young embryo sac (ES). The ES then elongates, its nuclei becoming located one in each pole.

Small vacuoles may develop above the apical nucleus, as well as below the basal one. Afterwards the first gametogenic division of the nuclei takes place. This division is synchronous (Fig. 2 K, L). The elongation of the ES continues. The top of the ES penetrates the tip of the nucellus and extends further up in the micropyle. At this point of development the apical epidermal cells of the nucellus break down, though remaining for a while as flattened accumulations at the margins of the ES. The breaking through the nucellus has usually taken place before the end of the first division in the ES (Fig. 2 L). At about the same time the epidermis surrounding the micropyle develops into a conspicuous integumentary tapetum. With the second gametogenic division the ES contains eight nuclei and subsequently becomes organized into a normal ES with three, not particularly large, antipodes (Fig. 2 N). Later the polar nuclei move towards the egg apparatus and take up a position immediately below it.

The ES development is bisporic in about 90% of all cases studied. A tetrasporic type of development is assumed to occur for the remaining

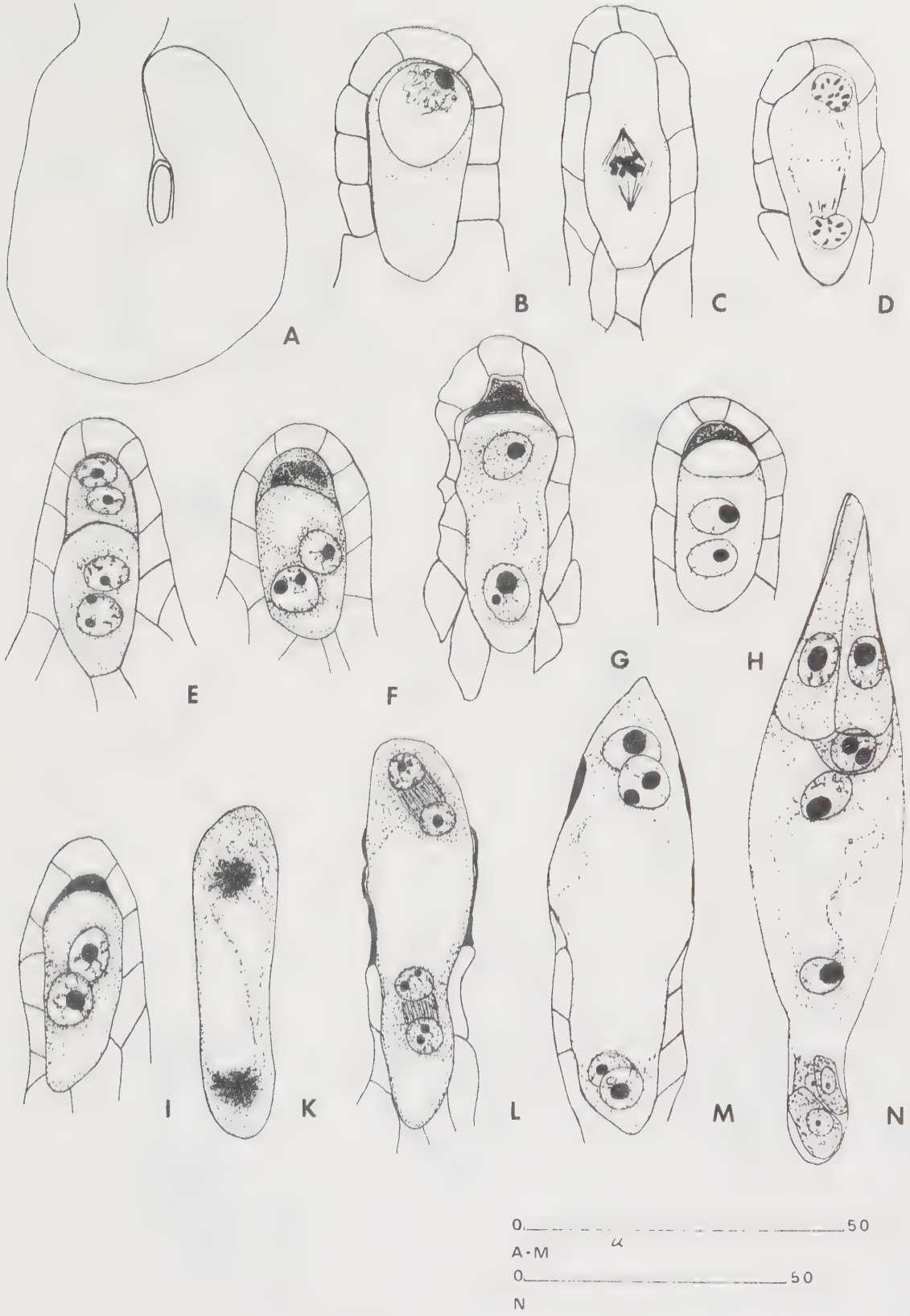

Fig. 2 Macrosporogenesis and female gametogenesis in Chaenactis artemisifolia. A. Young ovule. B. EMC. C. Metaphase of the heterotypic division of the EMC. D. Telophase of the same division. A partition wall is laying down. E. Two bivalent CMS. F—I. Different stages in the degeneration of the upper CMS and the enlargement and vacuolization of the lower one. K. A binucleate ES with a large vacuole. The nuclei in division. L. A later stage of the same division. M. Four-nucleate ES. N. Eight-nucleate, organized ES.

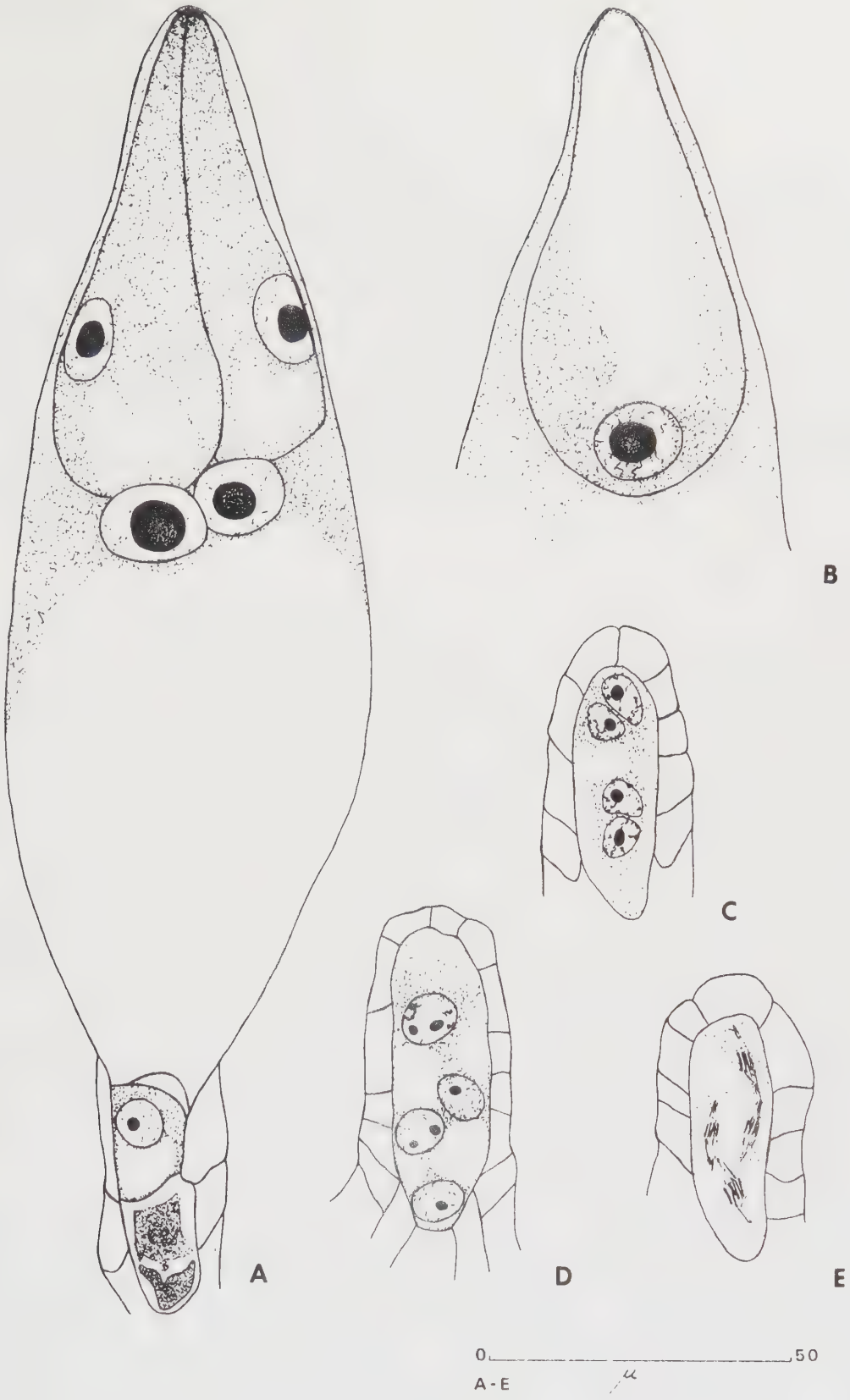

Fig. 3 Chaenactis artemisifolia. A. Eight-nucleate ES with polar nuclei side by side near the egg apparatus. B. The egg cell from A. C. Quadrivalent CMS. D—E. Four-nucleate ES of tetrasporic origin.

10%, based on the presence of nucelli, which consist of a single, centrally-placed, four-nucleate cell and epidermis (Fig. 3 C, D). The lack of all degenerate derivatives inside the epidermis and the presence of more or less distinct vacuoles indicate that the cell in question is really a quadrivalent CMS, on the point of transformation into a young four-nucleate ES. Such quadrivalent CMS were found in the same specimens as the bivalent ones. Although a large amount of material has been studied, it was not possible to follow the further course of development of the quadrivalent CMS.

2. *Chaenactis glabriuscula* DC. (Fig. 4).

The archespore is one- or two-celled (Fig. 4 A, B). In the latter case, however, one of the cells is very small giving the impression of being merely latent. Following the heterotypic division a cell wall develops (Fig. 4 C). The homotypic division results in linear tetrad of four cells (Fig. 4 D). All the macrospores show a tendency to germinate, as well as some tendency to enlarge, but the three upper ones are soon surpassed by the basal one. The three upper cells soon degenerate, the two middle ones usually a little faster than the apical one (Fig. 4 E, F). The ES enlarges and elongates and breaks through the nucellus. An integumentary tapetum starts to develop. The first division in the ES takes place and a binucleate ES is formed (Fig. 4 G). One nucleus becomes located in each pole of the sac, with an intervening vacuole. There is also a vacuole at the basal end of the ES. Only remnants of the nucellus are now visible. The two subsequent divisions give rise to an ES with eight nuclei (Fig. 4 H, I). The newly-formed, organized ES consists of an egg apparatus, two polar nuclei and two antipodes. The latter lie on top of one another along the ES-axis, occupying almost the whole of the basal half of the newly-organized ES (Fig. 4 K). The upper antipode is binucleate and larger than the lower, uninucleate one. The polar nuclei then fuse to form a large secondary nucleus situated in the neighbourhood of the egg

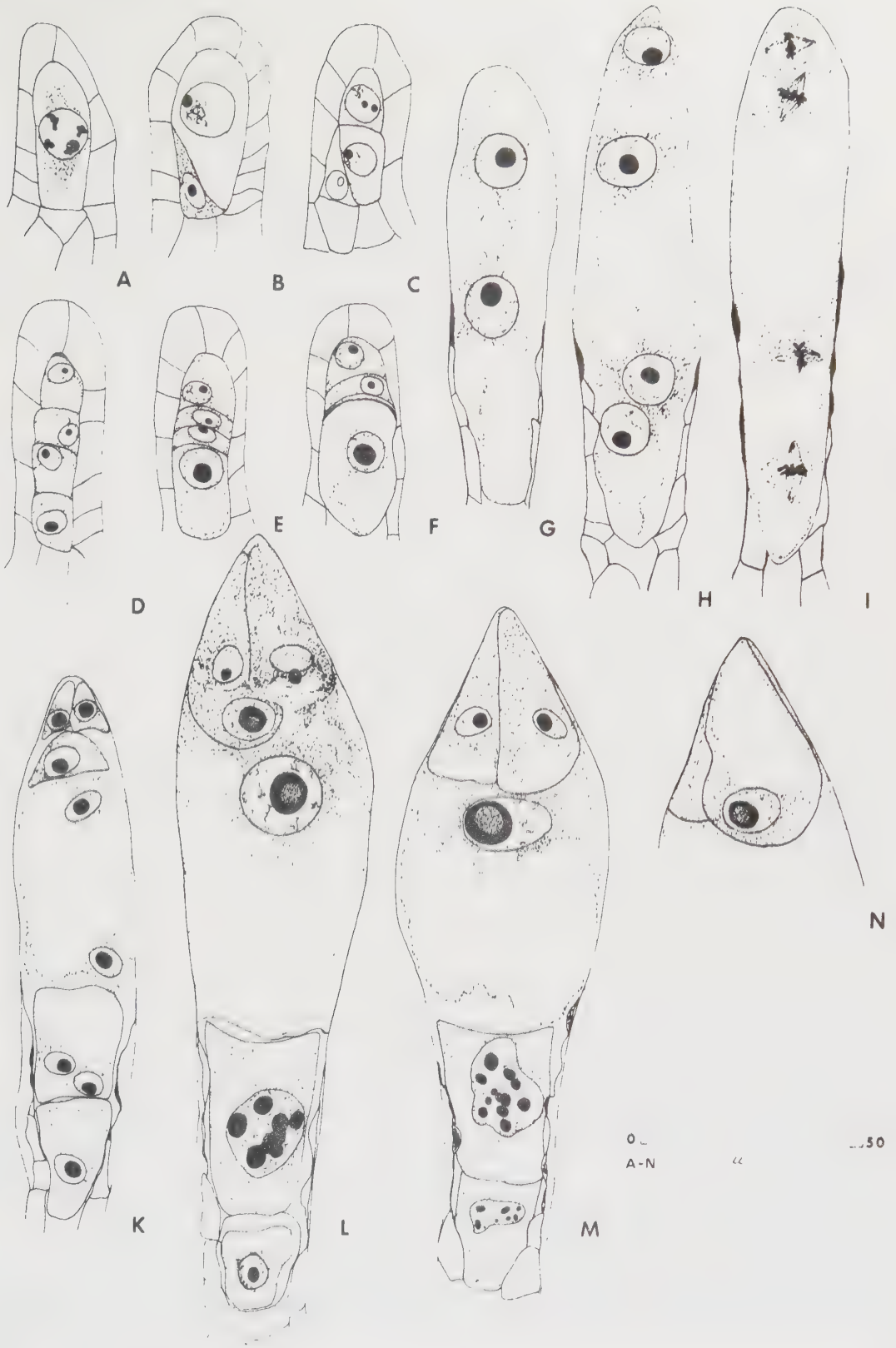

Fig. 4 Macrosporogenesis and female gametogenesis in Chaenactis glabriuscula. A. EMC. B. EMC and second archespore cell. C. Dyad cells with second archespore cell showing. D. Linear tetrad. The plasma of the cells is vacuolized. E. and F. Different stages in degeneration of the upper three macrospores and enlargement of the lower one. G. Binucleate ES. H. Four-nucleate ES. I. The four nuclei in division. K. Organized eight-nucleate ES with two antipodes, the upper one binucleate, the lower one uninucleate. L. The upper antipode with a large synkaryon. The two polar nuclei have fused. M. ES with synkaryons in the antipodes. N. The egg cell from M.

apparatus (Fig. 4 L). At this stage in the development a large, more or less lobate nucleus, with several nucleoli, appears in the upper antipode. Later on the nucleus of the basal antipode also develops into a multinucleolate one, somewhat increased in size and also of somewhat irregular contour (Fig. 4 M).

3. *Chaenactis Fremontii* A. Gray (Fig. 5).

The archespore is one-celled (Fig. 5 A). The meiosis results in a linear tetrad of four macrospores. The two middlemost ones degenerate early. The apical tetrad cell shows definite signs towards germination, but degenerates without further development. The basal macrospore alone forms the definitive ES. It soon becomes enlarged and vacuolated, and the nucleus divides to form a binucleate ES (Fig. 5 C). The two daughter nuclei are at first located in the apical part of the expanding ES. The usual bipolar position of the nuclei is first attained after a more pronounced elongation of the ES has occurred. As the ES elongates, rupturing the nucellus, an integumentary tapetum starts to develop. The nuclei of the ES then divide, to give rise to a four-nucleate ES (Fig. 5 E).

Through a third division an eight-nucleate ES is formed (Fig. 5 F), which becomes organized into an egg apparatus, two polar nuclei located in the central part of the sac, and usually two antipodes. The upper antipode is binucleate and larger than the lower, uninucleate one. The ES is thus of the same type as that of *Ch. glabriuscula*. The antipodal nuclei also usually behave in exactly the same manner as in the latter species, i.e. they undergo secondary divisions and fusions. In *Ch. Fremontii*, however, a variable number of antipodal cells may occur. As a result of secondary divisions up to five antipodes may be present in the mature ES (Fig. 5 L).

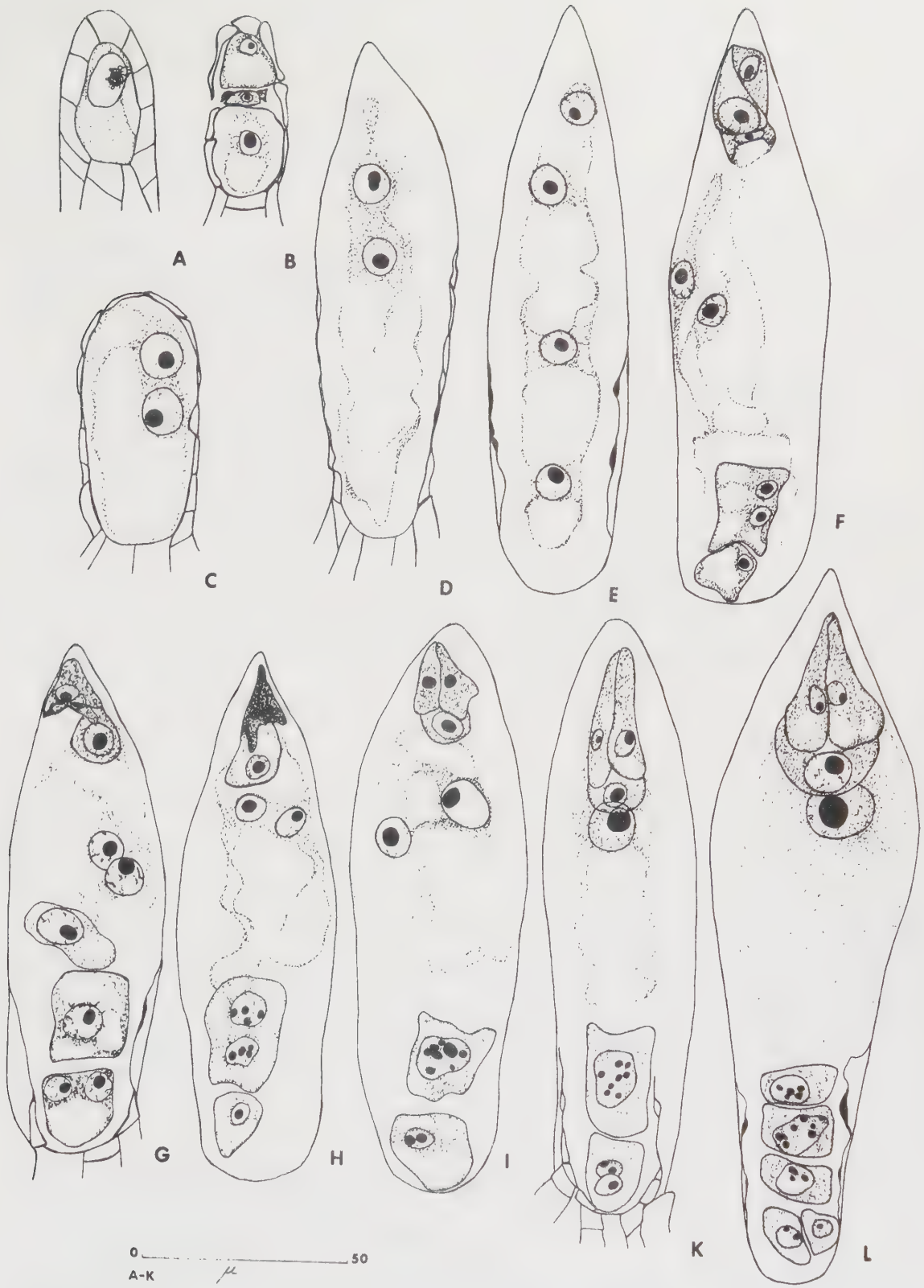

Fig. 5 Macrosporogenesis and female gametogenesis in Chaenactis Fremontii. A. EMC. B. A linear tetrad with the two middlemost cells almost degenerated. C. The nucleus of the basal macrospore has divided and a binucleate ES is formed. D. Binucleate ES. E. Four-nucleate ES. F. Eight-nucleate ES with two antipodes of which the upper one is binucleate, the lower one uninucleate. G. ES with the two upper antipodes uninucleate and the lower one binucleate. H—L. Different stages of development within the antipodes.

GENERA OTHAKE RAF. AND PALAFOXIA LAG.

The embryology of the genera *Othake* and *Palafoxia* is here dealt with together. Both genera are found in the southern United States and Mexico. In all, about a dozen species have been described.

Two species of the genus *Othake* were examined: *O. texanum* (DC.) Bush and *O. sphacelatum* (Nutt. ex Torr.) Rydb., together with *Palafoxia linearis* Lag. The material of the genus *Othake* was grown in Uppsala Botanical Garden and in the Hortus Bergianus, Stockholm, in 1962 and in the Göteborg Botanical Garden in 1972 and 1973, from achenes obtained from Uppsala. The ultimate origin of the achenes is unknown. Fixed material of *Palafoxia linearis* was obtained from Dr Peter H. Raven (herbarium number 17199) and of *Othake texanum* by Dr Örjan Nilsson, Uppsala.

A. Microsporangium, Microsporogenesis and Male Gametophyte (Fig. 6).

The two genera *Othake* and *Palafoxia* are treated together, since their development is practically identical. The cell layers of the sporangium wall, the development of the PMC and their derivatives and of the tapetum are more or less identical with the conditions found in *Chaenactis*. However, there are a few minor deviations. The nuclei of the tapetum undergo their transformations a little earlier, relative to the development stages of the PMC, than in *Chaenactis* (cf. Fig. 6 B with 1 B).

B. Macrosporogenesis and Female Gametophyte.

1. *Othake texanum* (DC.) Bush (Figs. 7, 8).

The archespore is one-celled. Development is generally tetrasporic (Fig. 7 A—D). Meiosis results in a four-nucleate CMS. The nuclei are separated by a large vacuole that occupies the entire central part of the cell. One

nucleus is located at each pole and the other two lie opposite to each other in the central part of the cell (Fig. 7 G). Besides the development just described, a monosporic development (Fig. 7 H—L) was also observed in the same specimens, in about 20% of all cases. The further development of the monosporic ES has not been studied.

In one instance a confusing configuration was found (Fig. 7 M). Three vacuolated cells lay in a single row inside the nucellar epidermis. The two basal cells are binucleate, the apical one four--nucleate. The cells are separated by "abnormal" walls. Whether the original state was a true tetrad, in which the walls between the two uppermost cells had broken down, or a triad, is impossible to say. The cell walls present, however, appear to be in process of disintegrating. Since this configuration has been seen only once, too much attention should not be paid to it. It may be a rare abnormality, very divergent from the normal development pattern.

Fig. 7 N shows a four-nucleate ES. The nucellus has degenerated. The nuclei are arranged more or less linearly, in two pairs separated by a large vacuole. In other cases (Fig. 7 G, O) the nuclei were arranged in a 1+2+1 pattern.

In the tetrasporic ES the two lower nuclei of the four-nucleate ES usually take little or no part in the subsequent development (cf. Fagerlind 1944, Harling 1950, 1951 a, b). As a result, in the stages which correspond to the 8-nucleate and 16-nucleate ES of the normal development scheme instead an ES with a reduced number of nuclei in the basal part and with various other abnormities is often encountered.

Fig. 8 A shows an ES, with two apical nuclei and six metaphase-spindles at the opposite pole. Hence the divisions at the apical and basal poles are asynchronous, those at the apical pole being relatively delayed. The organized ES shown in Fig. 8 B is almost certainly at an early stage. Neither the synergids nor the egg cell have attained their final shapes and sizes (cf. Fig. 8 B with 8 C—G). Below the lower polar nucleus there are seven free nuclei, some of which are very small. The fully-organized ES always posesses a normal egg apparatus and normal polar nuclei (Fig.

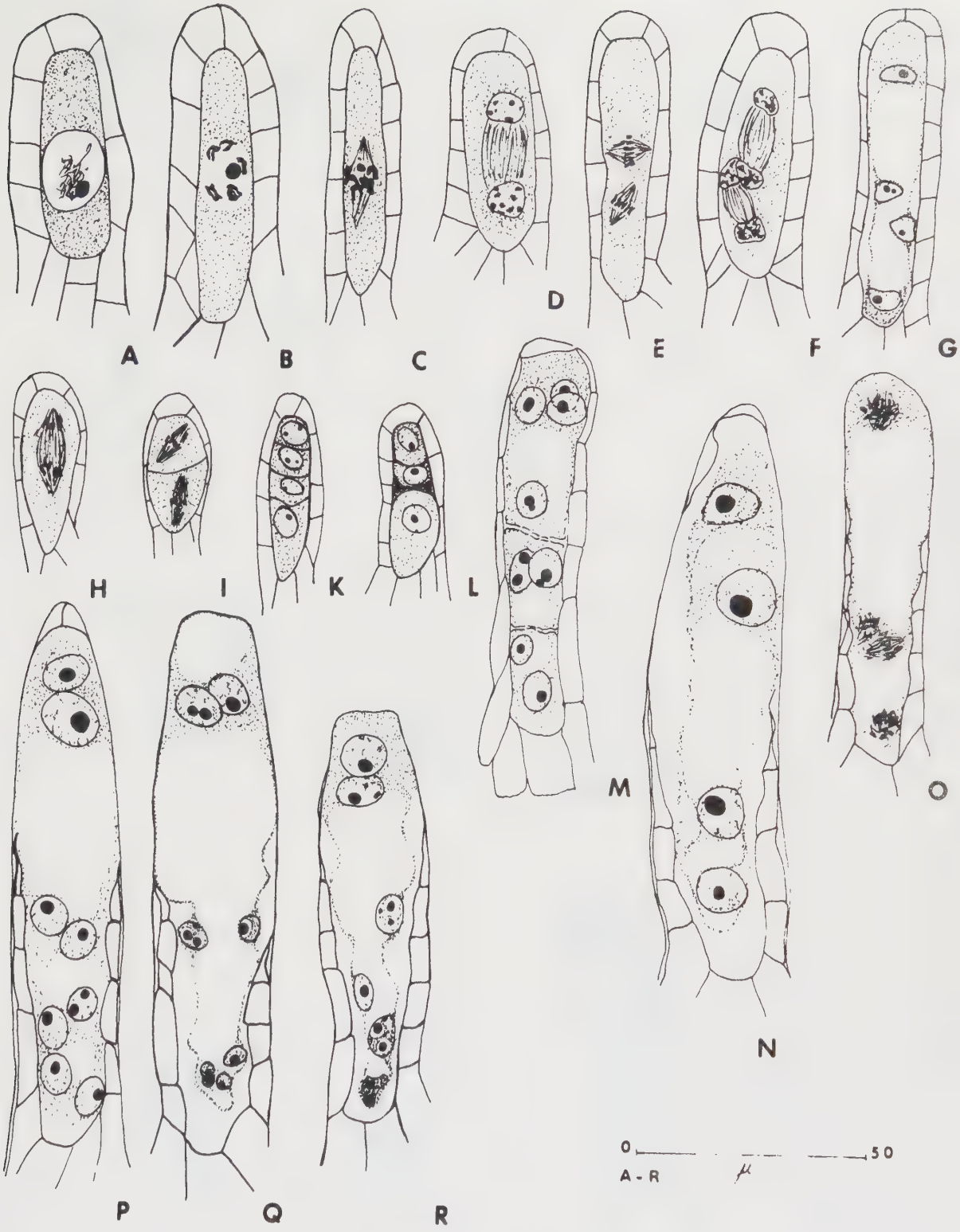

Fig. 7 Macrosporogenesis and female gametogenesis in Othake texanum. A and B. EMC in prophase. C and D. Ana- and telophase stages of the heterotypic division. E and F. Meta- and telophase stages of the homotypic division. G. Four-nucleate ES of tetrasporic origin. H. EMC in division. I. The nuclei of the dyad cells in division. K. Linear tetrad. L. Elongation of the basal macrospore and degeneration of the upper three. M. Probably a transition form between mono- and tetrasporic development. N. Four-nucleate ES. O. The four nuclei in division. P, Eight-nucleate ES. Q. ES with nuclei or different sizes. R. ES with six nuclei.

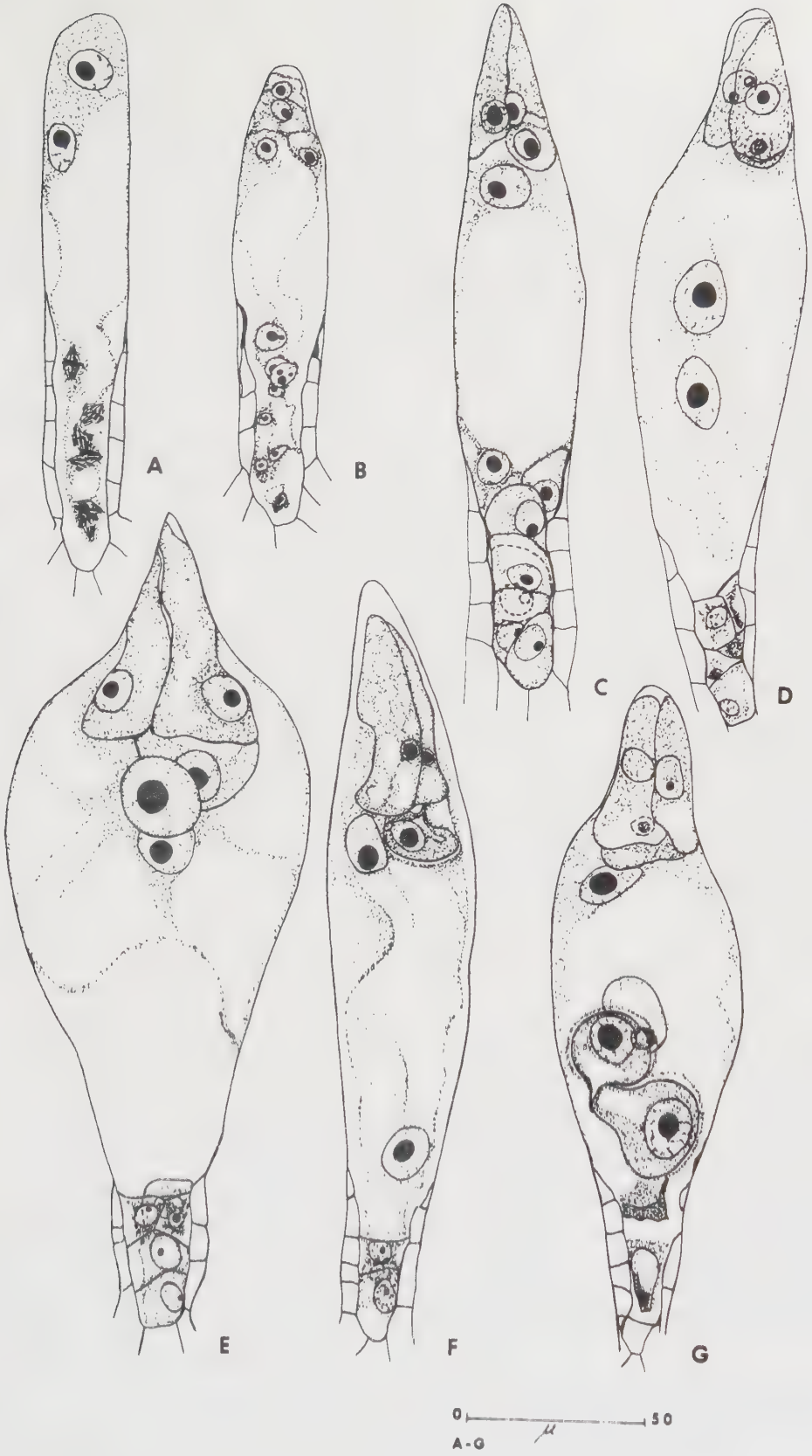

Fig. 8 Othake texanum. A. Eight-nucleate ES with six of the nuclei in division. B. ES with twelve nuclei. C. Organized ES with six antipodes. The polar nuclei located one in each end of the sac. D. Later stage with the polar nuclei in the central part of the sac. Five antipodes. E. The polar nuclei have moved towards the egg-apparatus. Five antipodes. F. ES with three antipodes. G. ES with two of the antipodes shaped as egg-cells.

8 C—G). In contrast the conditions in the antipodal system are very variable. From three to six distinct antipodal cells were found in the material studied (Fig. 8 C—G). Very probably some of the cases represent monosporic ES and some tetrasporic ones. Because of the previous, more or less pronounced, nuclear passivity and/or degeneration, tetrasporic ES with 11 antipodal cells can hardly be expected.

Another peculiarity found in *O. texanum* is illustrated in Fig. 8 G. Close to the lower polar nucleus and the degenerate antipodal cells arranged in a quite normal manner, we find two cells of deviant shape. They project into the ES like large, pear-shaped vesicles. Their slender, basal, peripheral part contains a large vacuole, while their more spacious apical part contains a very large nucleus. These remarkable configurations very much resemble those of egg cells. They are to be considered as re-shaped antipodal cells. In normal cases, too, in *O. texanum*, the antipodes show a tendency to be long-lived and to exhibit secondary growth to different degrees (cf. Fig. 8 C, D). During all the stages illustrated in Figs. 7 N—R and 8 A, and in later stages, an integumentary tapetum is present.

2. *Othake sphacelatum* (Nutt. ex Torr.) Rydb. (Figs. 9, 10).

As an aberration, two ovules were once found in a single ovary (Fig. 9 A), orientated with their micropyles facing one another.

The archespore is one-celled. Neither the heterotypic (Fig. 9 B—E, nor the homotypic division (Fig. 9 F) was followed by wall formation between the daughter nuclei, i.e. development is tetrasporic. After vacuolation, i.e. after the germination of the quadrivalent macrospore, the nuclear constellation tends to be 1+2+1.

After the first gametogenic division the tetrasporic ES usually possesses only six nuclei (Fig. 9 I). As a rule two nuclei, of normal size and showing no remarkable nucleolar conditions, are situated at the apical pole of the ES, sometimes beneath a small vacuole. The four nuclei at the chalazal end frequently possess more than one nucleolus. They are also smaller. Probab-

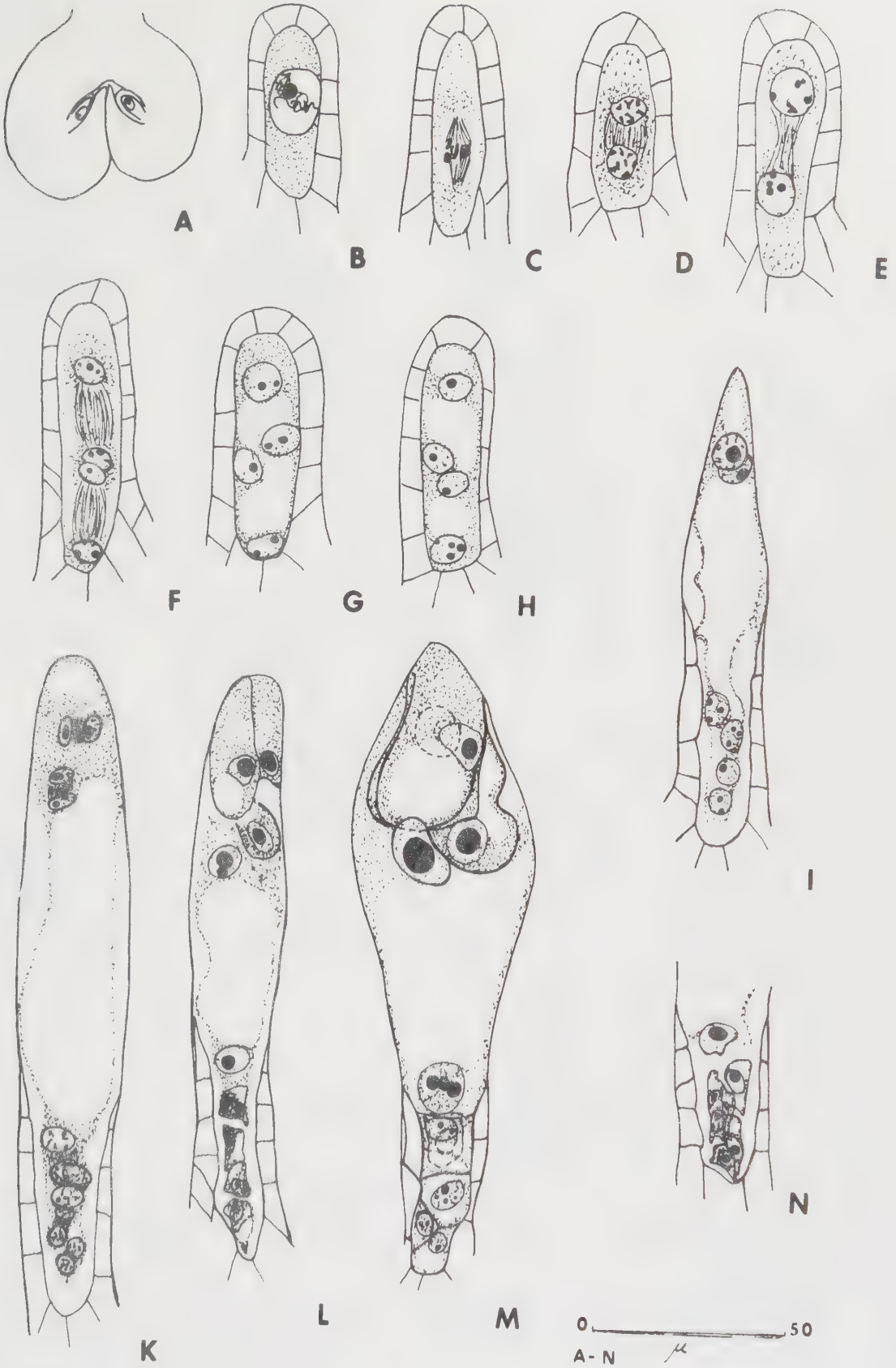

Fig. 9 Macrosporogenesis and female gametogenesis in Othake sphacelatum. A. Abnormal condition with two ovules. B. EMC. C-E. Meta-, early and late telophase stages of the heterotypic division of the EMC. F. Telophase stage of the homotypic division. G and H. CMS with four nuclei. I. ES with six nuclei. K. Newly divided ES with twelve nuclei. L. Organized ES with four antipodes. The polar nuclei located one in each end of the sac. M. ES with five antipodes. N. Basal part of an ES with six antipodes.

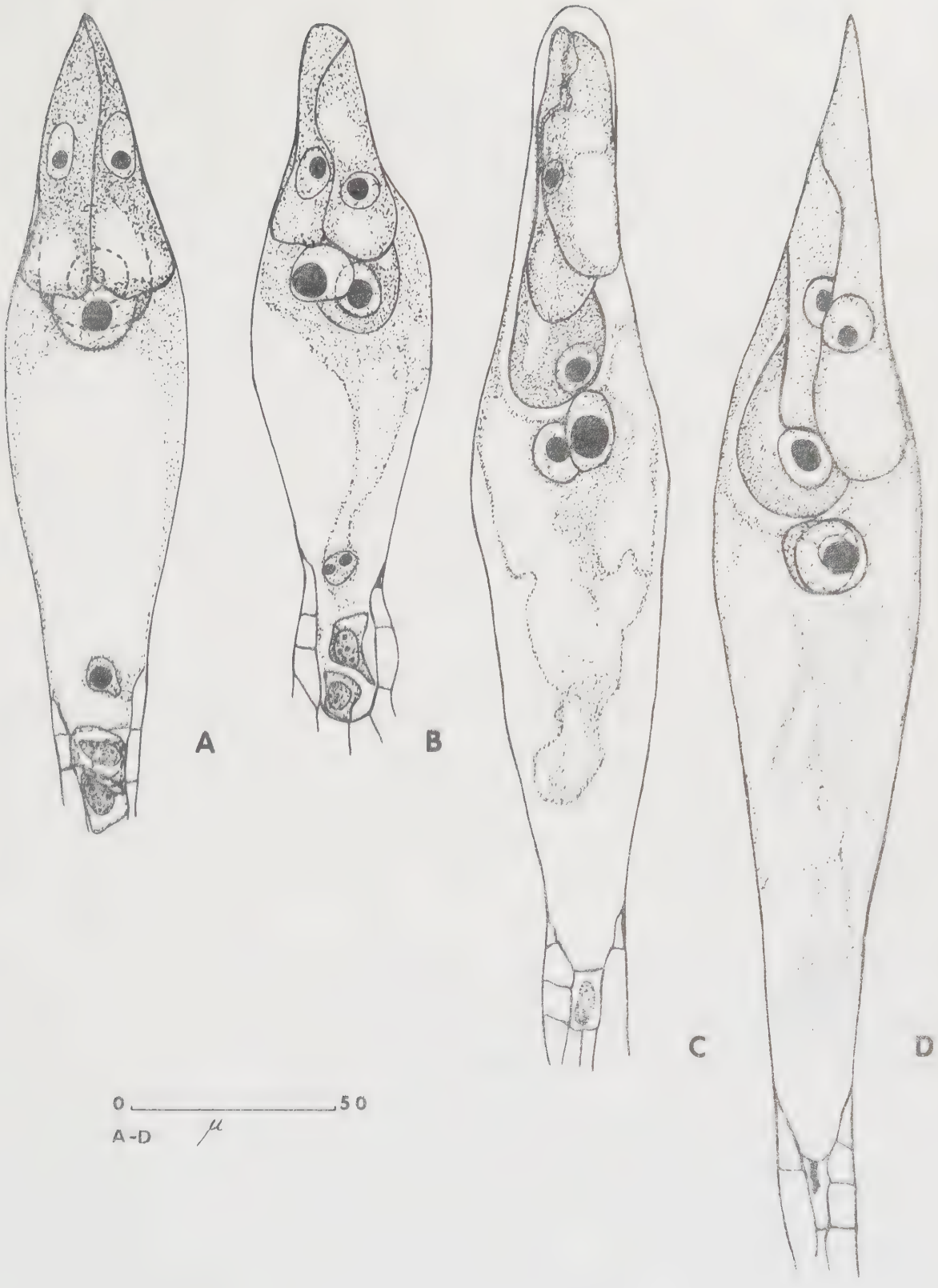

Fig. 10 Othake sphacelatum. A. ES with three antipodes. B. ES with two antipodes. C. The polar nuclei located close to the egg apparatus. Only one antipode. D. Later stage with one degenerating antipode.

ly only the uppermost of the three lower ES nuclei shown in Fig. 9 G—H, has in fact divided. The remaining nuclei either remained passive, or their normal division was interrupted by the formation of restitution nuclei, etc. Sometimes these nuclei may, however, divide normally in the second gametogenic division (Fig. 9 K).

In a fully organized ES a variable number of antipodal cells are present. The early stages often possess four, five or six, uninucleate antipodes (Fig. 9 L—N), while in older stages the antipodal cells decrease in number (Fig. 10 A, B) and in yet older stages they are often only represented by degenerate remnants, or are completely absent (Fig. 10 C, D).

In *O. sphacelatum*, too, an integumentary tapetum forms already during the four-nucleate ES stage.

3. *Palafoxia linearis* Lag. (Fig. 11).

Only a limited amount of material of this species was available for study. Therefore only a few stages of the ES-development can be described. The nucellus and archespore are of the common type (Fig. 11 A—B). The heterotypic division results in a binucleate cell, i.e. without wall formation (Fig. 11 C—D). A well-developed integumentary tapetum is present.

An eight-nucleate ES was found in a single case (Fig. 11 E). Two large nuclei were situated at the apical pole, and six small nuclei basally beneath the central vacuole. Two of the basal nuclei had a somewhat deviant shape and contained two nucleoli. The basal nuclei were partly separated from each other by two rather spacious vacuoles.

Two organized ES were also observed. One of them was eight-nucleate with three antipodes. The apical polar nucleus was much larger than the basal one (Fig. 11 G). The other organized ES (Fig. 11 F) differed considerably, but the scarcity of material allows few conclusions to be drawn. The ES shown in Fig. 11 E has certainly been preceded by an ES with a large apical nucleus and three smaller basal nuclei. Such a constellation is cha-

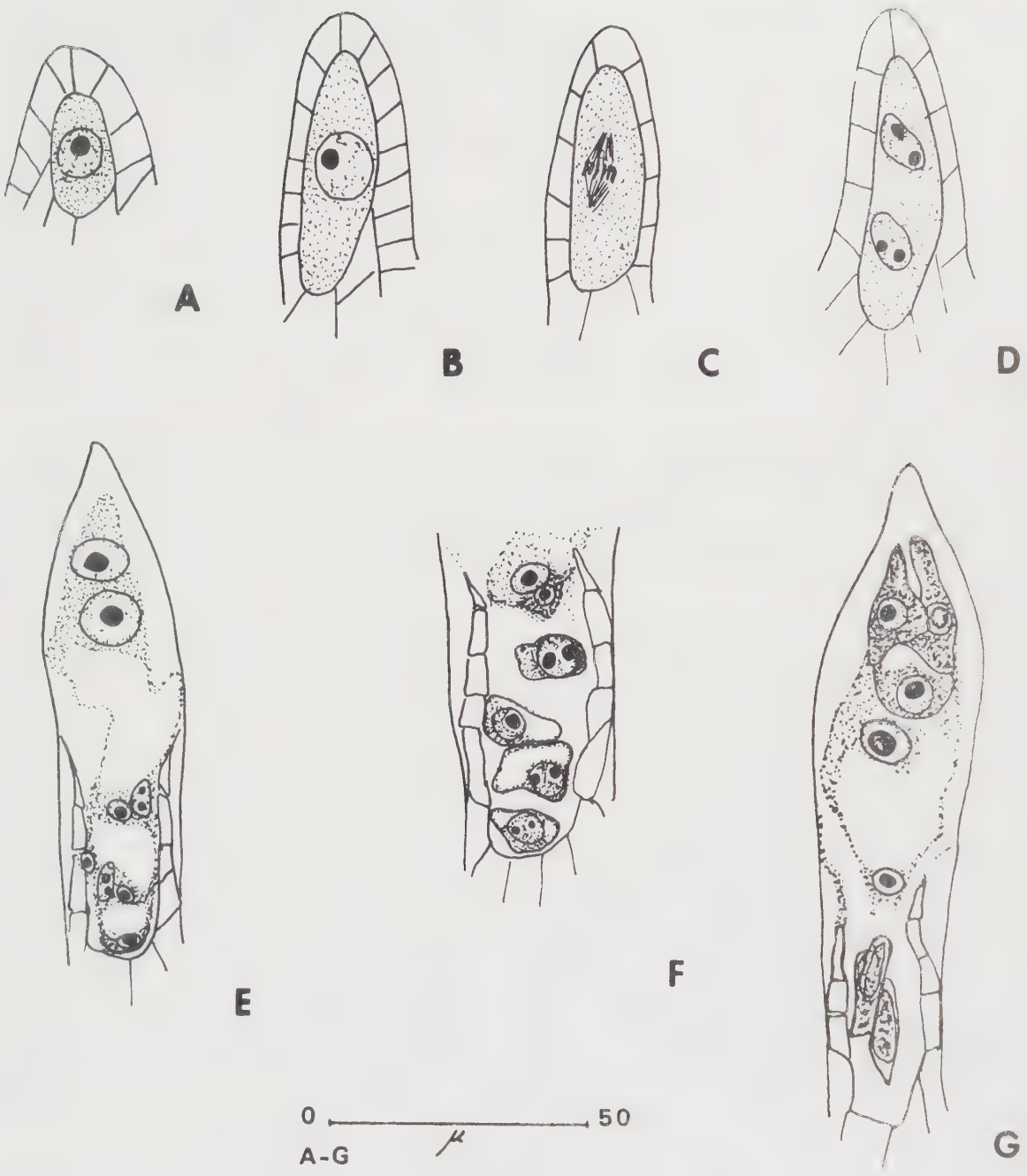

Fig. 11 Macrosporogenesis and female gametogenesis in Palafoxia linearis. A and B. EMC. C. EMC in division. D. Dyad stage. E. Eight-nucleate ES. F. Basal part of organized ES with four antipodes. G. ES with three antipodes.

racteristic of certain tetrasporic ES types. The ES shown in Fig. 11 D and E suggest that ES development is indeed tetrasporic in *P. linearis*.

GENUS MONOLOPIA DC.

Monolopia is an endemic Californian genus which according to Crum (1940) constitutes a natural group of four species. The species examined here is *M. gracilens* Gray. The investigation is based on fixed material supplied by Dr Peter H. Raven, St. Louis, Missouri, U.S.A.

A. Microsporangium, Microsporogenesis and Male Gametophyte (Fig. 12).

The development of the microsporangium in *Monolopia* is in general the same as in the above-mentioned genera. However, some deviations should be mentioned.

The rounding-off and separation of the PMC start rather early. In relation to the onset of meiosis, the secondary development involving the tapetal nuclei also occurs very early. In contrast to these phenomena the tapetal components are discernable as distinct, separate cells, up to the stage at which the young microspores are quite free from one another and possess an immature exine. At this stage much of the tapetal cytoplasm becomes interspersed with the young microspores, as thin veils (Fig. 12 E). When the microspores have developed into two-celled gametophytes, with a well-developed exine, a periplasmodium is still present (Fig. 12 F), but somewhat later when the protuberances of the pollen appertures have become distinct, all traces of it have been eliminated (Fig. 12 G).

B. Macrosporogenesis and Female Gametophyte (Fig. 13).

The archespore is one-celled (Fig. 13 A). Meiosis results in a four-celled, linear tetrad (Fig. 13 B, C). Instead of the linear arrangement a so-called T-shaped tetrad is sometimes seen (Fig. 13 E). The chalazal macrospore is a little larger than the others and is the only one that is functional. The

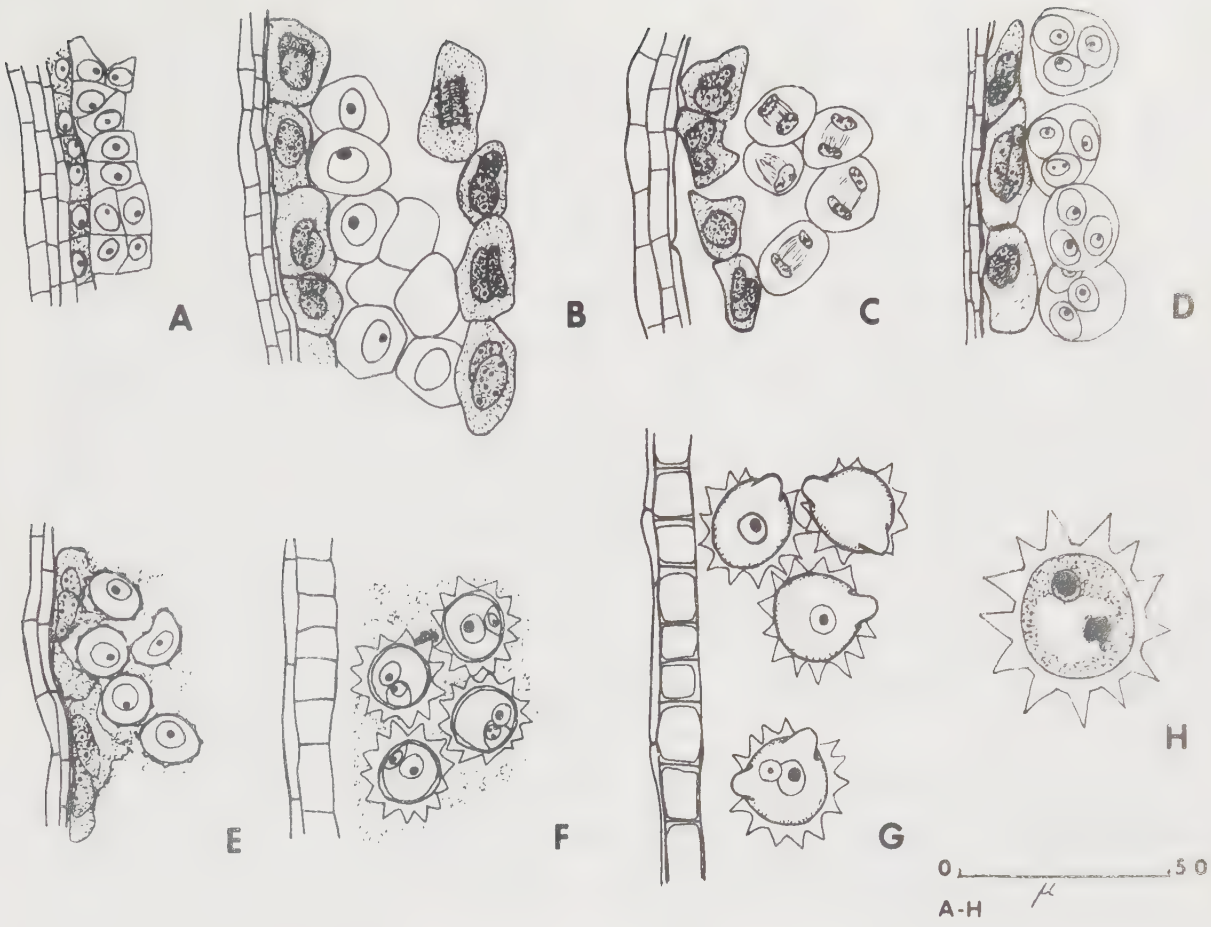

Fig. 12 Microsporogenesis and male gametogenesis in Monolopia gracilens. A young anther with PMC. The tapetal cells uninucleate. B. PMC with uni- or binucleate tapetal cells. C. Telophase of the heterotypic division. D. Pollen tetrads, The tapetal cells still uni- or binucleate. E. Young uninucleate pollen grains with just developing periplasmodium. F. Binucleate pollen grains. G. Pollen grains with spinuliferous exine. The periplasmodium has disappeared. The endothecium cells with wall thickenings. H. Ripe three-celled pollen grain.

three micropylar ones degenerate more or less rapidly. However, the apical cell or cells of T-tetrads remain alive for some time (Fig. 13 E—G).

The functional macrospore undergoes considerable expansion and vacuolation to become a young ES (Fig. 13 D—I). During the first gametogenic division the nucleus is situated more or less centrally in the ES, apparently forming the centre of a system of radiating, cytoplasmic lamellae. The two daughter nuclei are also centrally placed for a short time (Fig. 13 G—I). The daughter nuclei most probably become separated by a vacuole prior to the next division, but the material studied was insufficient to prove this with certainty. The four-nucleate stage is shown in Fig. 13 K. The nuclei are linearly arranged.

As in the other genera studied, the nucellar epidermis now becomes ruptured. The upper half of the ES is exposed in the micropyle, surrounded by a well-developed integumentary tapetum.

The four nuclei then divide to form an eight-nucleate ES (Fig. 13 L). Commonly during this stage, the nuclei in both the egg and antipodal zones are very much contracted, the polar nuclei alone retaining their normal appearance.

Young organized ES (Fig. 13 M—O), as well as fertilized ES bear witness to somewhat variable antipodal conditions. An antipodal system of three cells was sometimes found (Fig. 13 M). However, a system of two cells, with secondary nuclear divisions and fusions seems to be more common (Fig. 13 N—P). The lateral, sub-basal cell of the ES shown in Fig. 13 O represents quite a rare condition.

The development of the egg pole cytoplasm and of the polar nuclei is quite normal.

The *Monolopia gracilens* material included a few post-fertilization developmental stages. The young embryo is very soon formed by two superposed basal cells and an apical cell which will become transversely divided (Fig. 13 P). The endosperm is cellular at a very early stage (Fig. 13 P). The antipodes remain for a considerable time (Fig. 13 P—Q).

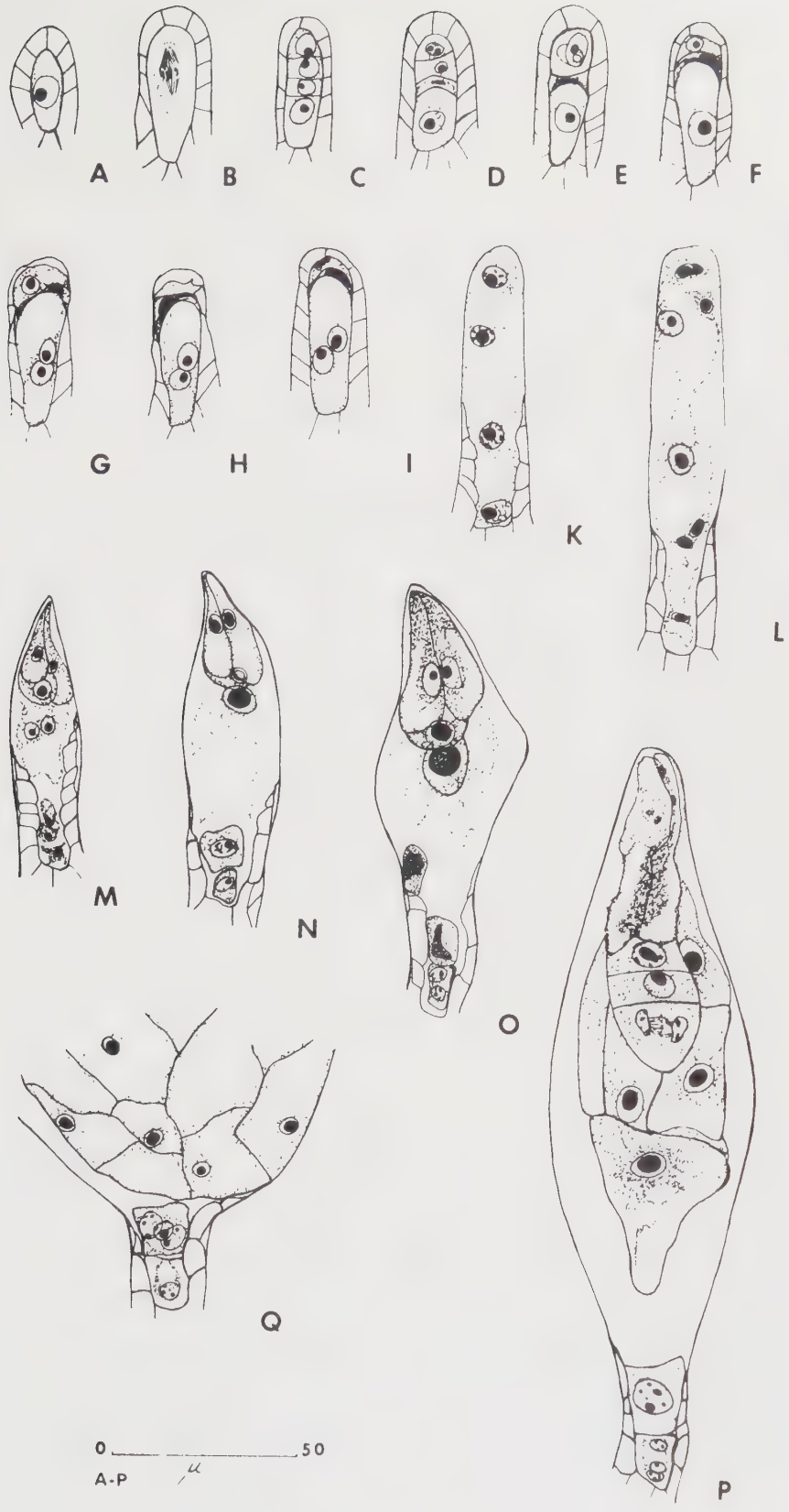

Fig. 13 Macrosporogenesis and female gametogenesis in Monolopia gracilens. A. EMC. B. EMC in division. C. A linear tetrad. D-F. Different stages in degeneration of the upper three tetrad cells and elongation of the lower, functional one. E. The tetrad cells in a T-shaped arrangement. G-I. Binucleate ES. The upper tetrad cells degenerating. K. Four-nucleate ES. L. An eight-nucleate ES. M. Organized ES with three antipodes. N. The same with two antipodes. O. ES with three antipodes one of which is binucleate. P. Early stage in the development of the embryo. Endosperm cells. Two antipodes, the basal one three-nucleate. Q. Chalazal part of an old ES. The antipodes still showing.

GENUS CADISCUS E. MEY.

Cadiscus is an endemic, monotypic genus from the Malmesbury District of South Africa. Fresh material (Hall 958) of the only species, *C. aquaticus* E. Mey., was examined.

A. Microsporangium, Microsporogenesis and Male Gametophyte (Fig. 14).

Development of the microsporangial wall, as well as of the sporogeneous tissue, was in general the same as that found in the other genera studied. Nevertheless, some comments on minor deviations may be of value.

At the stage when the PMC are discernable (Fig. 14 A), the cells of the tapetum are uninucleate, but remarkably large-sized and with a very dense cytoplasm. Their nuclear divisions start at this stage (Fig. 14 B). The PMC subsequently become free from one another but are enveloped by callose mantles, which locally develop emergent 'heels' (Fig. 14 C—D). During the tetrad stage and the liberation of the tetrad cells this mantle becomes transformed into a relatively even callose capsule (Fig. 14 E). At the same time contact between the tapetal cells is lost. These latter cells are then only locally attached to the sporangial wall. In the light microscope it is impossible to decide whether or not they are still surrounded by an intact cell wall. Only when the still uninucleate microspores have aquired a well--developed exine does the transformation of the tapetum into a true periplasmodium begin (Fig. 14 F). The tapetum first becomes transformed into a peripheral cytoplasmic layer, with distinct traces of the cell walls still visible. The nuclei are still peripherally located, but several finger-like processes from the tapetal cells intrude between the neighbouring microspores. During the stage characterized by two-celled pollen grains with a mature exine and apertures with a protruding intine, the periplasmodium is still present in the form of scattered nuclei and thin veils of a "lacuna"-rich cytoplasm which envelop the pollen grains (Fig. 14 G). A little later, the periplasmodium has become wholly resorbed (Fig. 14 H).

B. Macrosporogenesis and Female Gametophyte (Fig. 15).

The archespore is one-celled (Fig. 15 A—B). Macrosporogenesis results in

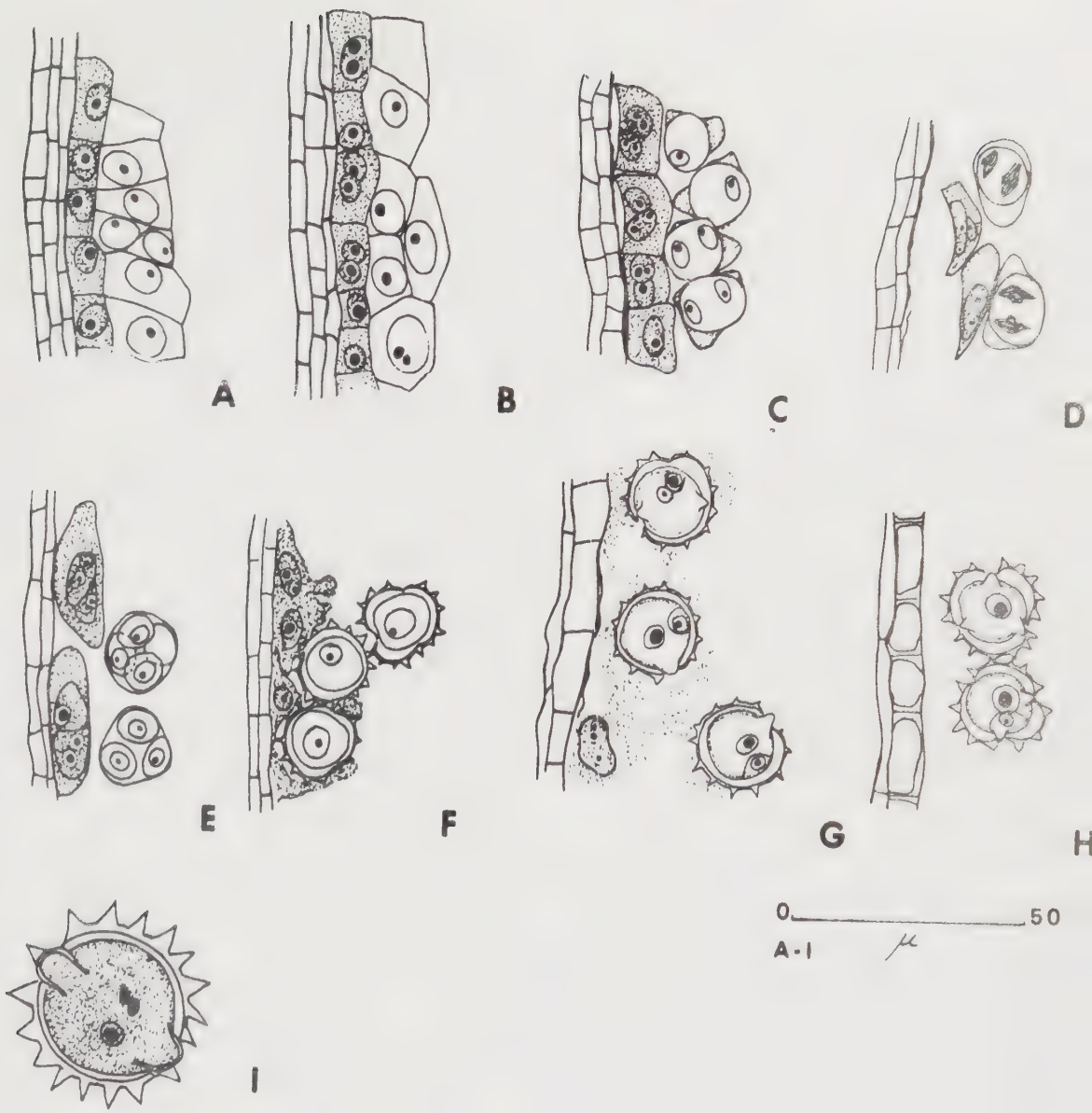

Fig. 14 Microsporogenesis and male gametogenesis in Cadiscus aquaticus. A. Early PMC stage with uninucleate tapetal cells. B. Later stage with uni- or binucleate tapetal cells. C. Pollen dyads. D. The homotypic division. The tapetal cells with one or two nuclei with more than one nucleolus. E. Pollen tetrads. F. Formation of the periplasmodium. Uninucleate pollen grains. G. Binucleate pollen grains. H. Triporate pollen grains with spinuliferous exine. Endothecium cells with wall thickenings. The periplasmodium has disappeared. I. Ripe three-celled pollen grain.

the formation of a linear tetrad (Fig. 15 B—D). In four of 21 tetrads observed a T-shaped arrangement was found (Fig. 15 E). The upper two cells disintegrate early on. The two lower ones become vacuolated and enlarge (Fig. 15 F). The ES development occurs according to the Normal type.

On two occasions I found two competing two-nucleate ES (Fig. 15 G), both of which had just penetrated the nucellar epidermis. More normally the sub-basal ES degenerates at an earlier stage, to leave the ES formed by the basal macrospore as the sole survivor.

When the ES has reached the four-nucleate stage, the nucellar apex has already become penetrated and an integumentary tapetum has developed. The four nuclei are grouped in a 2+2 position. The lower group is separated from the basal pole by a large vacuole(Fig. 15 H). A corresponding arrangement is retained when the two-groups develop into four-groups (Fig. 15 I). The basal vacuole then occupies about a third of the entire ES length.

In the fully organized ES the antipodal group always (Fig. 15 K—N) contains a basal cell with a single nucleus. This cell is crowned by a group of two or — after a secondary division — three, much smaller but still quite large, cells, which lie side by side, sometimes more or less obliquely or at least partially superposed (Fig. 15 K—N). By secondary nuclear divisions, all or some cells finally contain two or more nuclei. These divisions do not seem to be synchronous. In consequence both uni- and binucleate cells are very often present in the antipodal system. The basal antipode seems to remain uninucleate until the two polar nuclei have fused (Fig. 15 L). In the cases studied the nucleus of the basal antipode then divides twice to yield a four-nucleate antipode with the nuclei located in the central part of the cell (Fig. 15 M). A stage shortly after fertilization can be seen in Fig. 15 N. Even at this late stage only four nuclei are found in the chalazal antipode.

Fig. 15 Macrosporogenesis and female gametogenesis in Cadiscus aquaticus. A and B. EMC. C. Dyad cells in division. D. A linear tetrad. E. A T-shaped tetrad. F. The upper two tetrad cells degenerating. The lower ones with vacuoles. G. Two competing binucleate ES. H. Four-nucleates ES with a large basal vacuole. I. Eight-nucleate ES. K. Organized ES with three antipodes; the basal one occupies 1/3 of the length of the sac. L. The polar nuclei have fused and the fusion nucleus is located in the neighbourhood of the egg apparatus. Four antipodes. M. ES with the basal antipode four-nucleate. N. Later stage. The basal antipode still four-nucleate.

DISCUSSION

According to the results of my study several different ES-types are found in the Helenieae-Heleninae. The ES is monosporic and follows the Normal type in *Chaenactis Fremontii, Ch. glabriuscula, Monolopia* and *Cadiscus*. In *Othake texanum* the same conditions are only occasionally encountered. Here a tetrasporic ES development according to a modified *Chrysanthemum parthenium* type is much more common. The latter type was also the only one observed in *Othake sphacelatum* and *Palafoxia*. ES-formation was bisporic in *Chaenactis artemisifolia* but a tetrasporic one was also observed. Rosén (1944) reported a tetrasporic ES-development, following the *Fritillaria*-pattern, in *Gaillardia*.

A bisporic ES-development is very rare in the Compositae. It has been observed in the tribe Astereae in a number of *Erigeron* species (Holmgren 1919, Carano 1921, Chiarugi 1927, Bergman 1942, Harling 1951 b), and in *Ammobium alatum* in the Inuleae (Avanzi 1948). In the Anthemideae the species of *Argyranthemum* — a subgenus of *Chrysanthemum* — constitute the only instance so far known of bisporic ES-development (Harling 1951 a, Borgen 1972). Hjelmqvist (1951) reported an interesting type of a bisporic development in *Tridax trilobata* in the tribe Heliantheae. The EMC nucleus divides without cell plate formation, which results in a binucleate cell. Only after the homotypic division are cell plates laid down and then only between the newly-formed daughter nuclei. A row of three cells, of which the middle one contains two megaspore nuclei, therefore results. In most cases this binucleate cell develops into an ES. In such cases the development is thus bisporic. In other cases a uninucleate megaspore is functional and development is monosporic. As the result of the present investigation *Chaenactis artemisifolia* can be added to the hitherto-known cases of bisporic development in the Compositae.

The type of ES formation is usually uniform within any one species and is very often also so for all species in the same genus. However, an increasing number of cases of variation in the type of ES formation, occurring even within the same species and often in material from a single specimen, have been recorded, e.g. in *Chaenactis artemisifolia*. As men-

tioned above, ES development is in most cases bisporic, of the Allium type, but a tetrasporic development also occurs, though to a lesser extent. Unfortunately it did not prove possible to discover whether or not the young tetrasporic ES observed really did develop into mature ES, but there is no good reason to doubt this assumption.

Such a combination of both bi- and tetrasporic ES-development in a single species has, in the Compositae, only been observed previously in the genus *Erigeron*. In *Erigeron alpinus* (Chiarugi 1927) as a rule the ES is tetrasporic of the *Chrysanthemum parthenium* type, but some bisporic ES of the *Allium* type were also observed. Harling (1951 b) observed the same phenomenon in *E. frigidus*, in which a tetrasporic development, with 16 or fewer nuclei, together with a bisporic development of the same kind as in *E. alpinus* took place. This type of combination is also very rare in other families. In his review of the variations in ES development Hjelmqvist (1964) mentions some plants with rudimentary ovules. According to Moissl (1941) in *Viburnum* the ES is monosporic in normal fertile ovules and tetrasporic in rudimentary ones. In certain species,however, viz. *V. acerifolium* and *V. lantana*, Suneson (1933) found that a bisporic development occurred in the fertile ovules. In *Adonis annua* (Bhandari 1962) ES development is in most cases bisporic, but in some sterile ovules development is tetrasporic.

As already mentioned, tetrasporic ES formation occurs in *Othake* and *Palafoxia*, in *Othake texanum* together with a monosporic one. Such a combination, however, is very rare in normally developed ovules. Bergman (1935) found it to occur exceptionally in *Leontodon hispidus*. Monosporic development was combined with cases where the walls between the macrospores were dissolved thus giving rise to quadrivalent CMS. Sharp (1912) found similar conditions in *Bletia Shepherdii*. In *Epidendrum variegatum* (Sharp op. cit.), *Epipactus pubescens* (Brown & Sharp 1911) and in the genus *Erigeron* (Harling 1951 b) ES formation could be monosporic, as well as bi- and tetrasporic. According to Donati (1913) the ES in *Euphorbia pulcherrima* could be mono- or tetrasporic. Similar variations have been described in the rudimentary ovules of some other plants, e.g. *Valeriana* (Asplund 1920) and *Clematis*

gaurana (Vijayaraghavan 1962), in all of which the ES development of normal, functional ovules is monosporic, of the Normal type, whereas in rudimentary ovules the walls between the macrospore nuclei become suppressed and development is tetrasporic (see also Fagerlind 1944).

Rydberg (1914) divided the genus *Chaenactis* into three sections. He named the sections after genera which are all now included in the genus *Chaenactis*. The perennial or biennial species were referred to the section Macrocarphus. On achene shape and the number and shape of the squamellae forming the pappus, when present, Rydberg divided the annual species into two separate sections: Acarphaea and Euchaenactis. He defined Acarphaea as follows: Achenes decidedly compressed, tapering below; plants scurfy when young; pappus, when present, of 8—10 lacerated squamellae. Euchaenactis was characterized by subterete achenes, linear or nearly so; plants floccose when young; pappus of 4 (rarely 5 or 6) principal, merely erose squamellae, with or without 1—4 shorter external ones.

Chaenactis artemisifolia was placed in the section Acarphaea and *Ch. glabriuscula* and *Ch. Fremontii* in the section Euchaenactis. In his revision of the genus *Chaenactis*, Stockwell (1940) did not subdivide the genus into different sections. The chromosome numbers (Bolkhovskikh et al.1969 published for the species in my study are: *Ch. artemisifolia* n= 8, *Ch. glabriuscula* n= 6 and *Ch. Fremontii* n= 5 (Stockwell 1940, Mooring 1965). Raven & Kyhos (1961) found a basic chromosome number of n= 6 in the perennial species they examined. Some tetraploids (n= 12) and hexaploids (n= 18), as well as diploids were also found. Among the annual species examined they recorded n= 5, 6, 7 and 8, the first two being found within the section Euchaenactis. According to these authors, *Ch. Fremontii* and *Ch. stevioides*, both desert annuals with n= 5, have clearly been derived from an ancestor with n= 6, possibly resembling *Ch. glabriuscula*, by unequal translocation and subsequent loss of a centric chromosome fragment. The counts n= 7 and n= 8 were found for species within the Acarphaea, which species were probably derived from ancestors with n= 6 by the addition of chromosome pairs.

The chromosome counts reviewed above substantially support Rydberg´s proposed subdivision of *Chaenactis*. The embryological differences support his subdivision into Acarphaea and Euchaenactis. Nevertheless, before a more definite conclusion can be reached, further studies are needed.

Rydberg (1914) and Ammerman Baltzer (1944) refer the genera *Othake* and *Palafoxia* to the subtribe Palafoxiinae ("Palafoxianae"). A third genus, *Polypteris*, is also placed in this subtribe. Many different interpretations of the generic relationships have been given and species have frequently been moved from one genus to another. According to the above-mentioned authors the group consists of three distinct, though closely-related, genera. Turner & Morris (1976), however, proposed recognition of only a single genus, *Palafoxia*, in agreement with Bentham & Hooker (1873), Hoffmann (1894), Cory (1946) and Shinners (1949). This proposal is based on morphology, biochemistry and chromosome numbers. The results of the present embryological study lend strong support to their opinion.

The chromosome numbers reported for the species in question are: *Othake sphacelatum* n= 12 (Smith 1965), *O. texanum* n= 11 (Turner 1960) and *Palafoxia linearis* n= 12 (Raven & Kyhos 1961). In the genus *Othake* a chromosome number of n= 10 has been found for two other species.

In *Cadiscus* ES formation and development is monosporic and of the Normal type but showing some remarkable differences in detail from the development found in other monosporic Helenieae-Heleninae. During the four-nucleate stage the nuclear constellation is of a special type, as is also the shape of the antipodal system (cf. Fig. 15). The antipodal model is almost certainly a direct result of the special nuclear constellation. Corresponding conditions are not found elsewhere in the Helenieae, but are present in other tribes of the Compositae. Both ES development and the structure of mature ES of *Cadiscus* fully agree with the conditions described by Afzelius (1924) for several *Senecio* species. Harling (1950) also found similar conditions in some Anthemideae-Anthemidinae genera.

Ornduff et al. (1967) found that the chromosome number of *Cadiscus aquaticus* was n= 10. The material they studied had been grown from achenes provided by Dr A.V. Hall, of the Bolus Herbarium at Cape Town. The collection number (Hall 958) indicates that the achenes originated from the same material which has been examined in the present embryological study. In the same paper (Ornduff et al. op. cit. pp. 211, 212) they state that n= 10 should be considered as the basic chromosome number for the tribe Senecioneae, with the following commentary: "The united uniseriate phyllaries, chromosome number and diagnostically typical styles leave no doubt that despite its aberrant pappus *Cadiscus* should be regarded as an aquatic derivative of the primary South African subtribe Othonninae of Senecioneae. This view is also logical on phytogeographical grounds, since the only other native members of Helenieae found in the Old World are *Flaveria australasica* Hook. of Australia and the endemic genus *Hypericophyllum* (Jauminae) of Africa". Raven & Kyhos (1961) have suggested the last-mentioned genus to be best placed in the Heliantheae, near the Coreopsidinae.

In a taxonomical study of the genus *Euryops*, Nordenstam (1968 pp. 31—32) expresses the opinion that "Ornduff et al. are probably correct in assigning the genus to Senecioneae-Othonninae, but the closer affinities of this little known genus should be further investigated. Characters of the involucre suggest a relationship to *Othonna*. The pappus of *Cadiscus* is characteristic, consisting of coarse, rigid, multicellular, scabrid bristles".

The result of my embryological investigations supports the relationship between *Cadiscus* and the Senecioneae-Othonninae proposed by the above mentioned authors, based on considerations of morphology, chromosome number and phytogeography.

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Embryological studies in Typhaceae, Sparganiaceae and Compositae

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The embryological development in *Typha* and *Sparganium* regarding the microsporogenesis and male gametogenesis as well as the macrosporogenesis and female gametogenesis, is very similar. Important characters such as the development of the tapetum, microspores, antipodes, and endosperm are entirely the same. The embryological characters together with previously known facts show that the order Pandanales in its usual circumscription is heterogeneous. The families Sparganiaceae and Typhaceae should be removed and put into an order of their own, the Typhales, with a single family, the Typhaceae, comprising two subfamilies, the Typhoideae and the Sparganioideae.

Five genera in the Helenieae-Heleninae (Compositae) have been examined: *Chaenactis* DC., *Othake* Raf., *Palafoxia* Lag., *Monolopia* DC. and *Cadiscus* E. Mey. The results of the embryological study support Rydberg's (1914) subdivision of the annual species of the genus *Chaenactis* into the two sections Acarphaea and Euchaenactis. Furthermore, the embryology reveals a close relationship between the genera *Othake* and *Palafoxia*, which supports the proposal by Turner & Morris (1976) to recognize only a single genus, *Palafoxia*. Embryologically the genus *Cadiscus* shows a close relationship with the Senecioneae-Othonninae.

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